VOLUME XXXV AUGUST, 1956 NUMBER 8

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

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The Journal of Clinical Investigation

Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, 622 West 168th St., New York 32, N. Y.

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Acceptance for mailing at special rate of postage provided for in paragraph (d-2), section 34.40, P.L. & R. of 1948, authorized February 13, 1950

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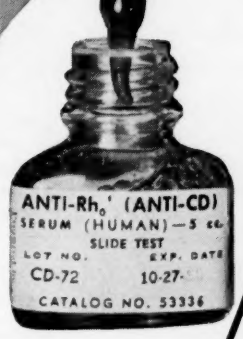

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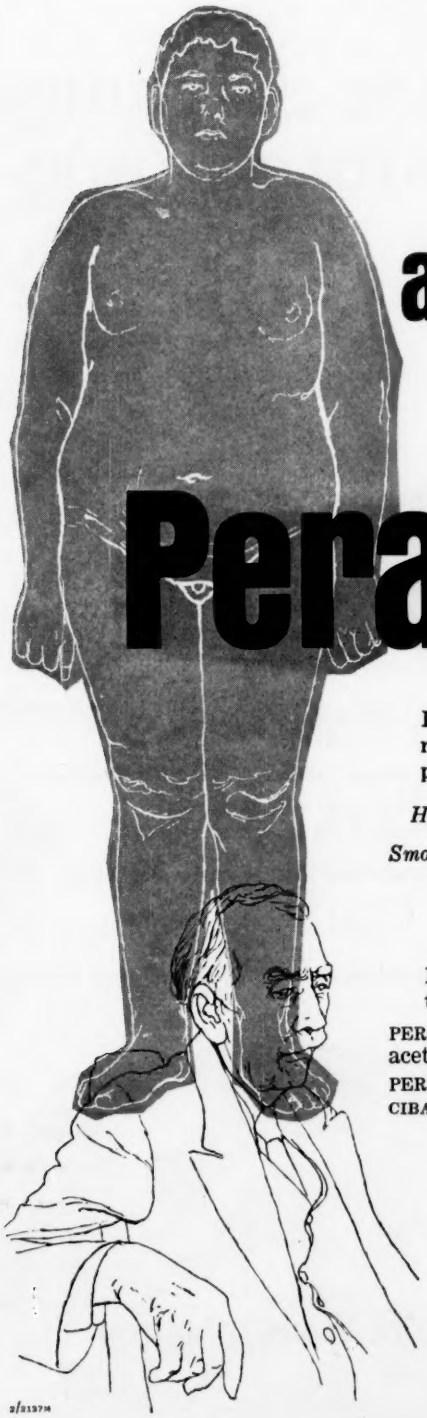

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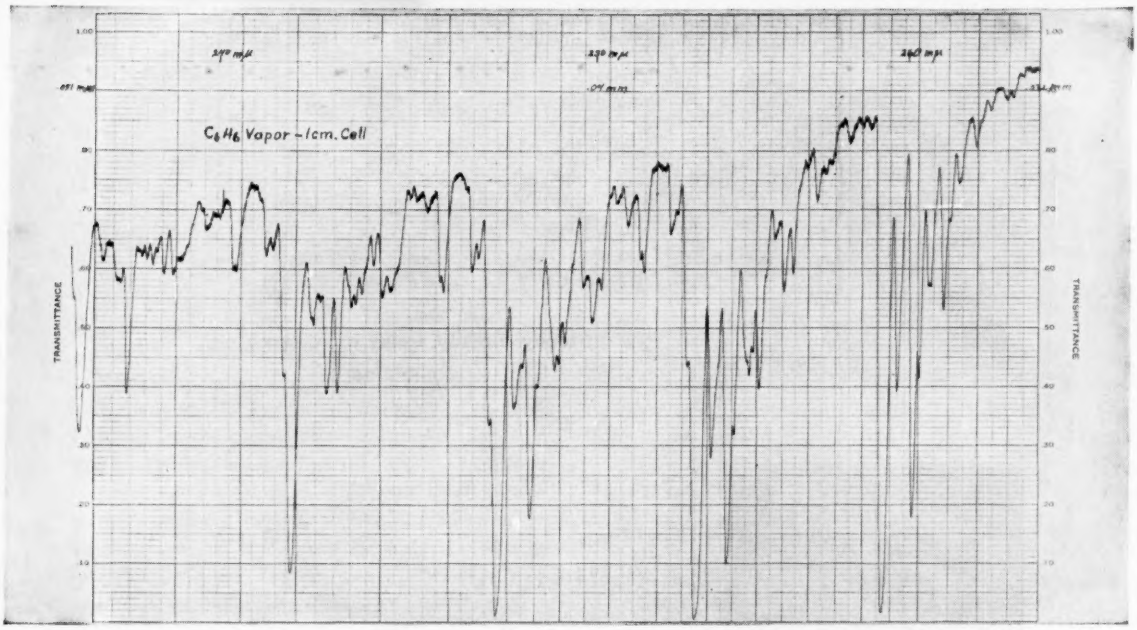

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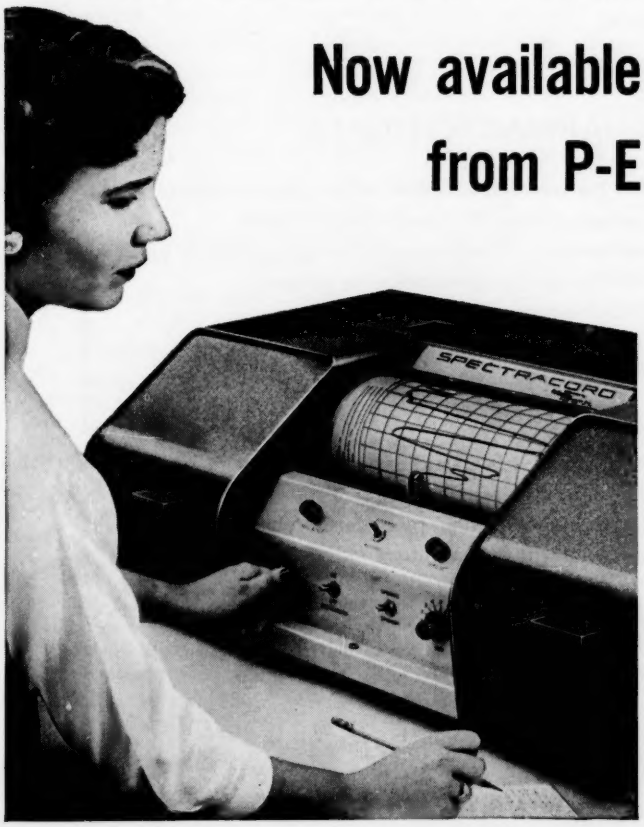

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**_Ibid._, p. 600.

THE INFLUENCE OF CHELATION ON RADIOMANGANESE EXCRETION IN MAN AND MOUSE [1]

By LAURENCE S. MAYNARD [2] AND SIDNEY FINK [3]

(From the Medical Department, Brookhaven National Laboratory, Upton, N. Y.; and the Division of Neoplastic Diseases, Montefiore Hospital, New York, N. Y.)

(Submitted for publication August 3, 1955; accepted April 3, 1956)

Manganese is capable of forming complex ions with many substances found naturally in the body (1). It seems reasonable to assume, therefore, that manganese injected as $MnSO_4$ (hereafter referred to as "ionic manganese") will be complexed in the body to a varying extent shortly after administration. If the Mn is strongly chelated before it is injected, a state of competition between ligands is set up, and an estimate of the strength of the physiological binding of manganese can be gained from the degree of alteration of the natural excretory pattern of the isotope resulting from the use of the strong chelate. In order to test this assumption, manganese was administered after previous chelation with an excess of EDTA [4] (hereafter referred to as "Mn chelate"), a strong complexing agent which has been shown to have pronounced effects on the excretion of some metals (2). The excretory patterns of "ionic" and "chelated" Mn were then compared.

When injected intravenously into experimental animals, manganese is rapidly removed from the circulating blood and distributed to various tissues of the body (3). The organs having the greatest capacity for manganese uptake are the liver, pancreas, and kidneys: and within the liver cell this element is concentrated to a high degree in the mitochondria (4). Excretion is almost entirely fecal, and more than half of this manganese is eliminated in the bile. Increasing the amount of manganese injected increases the rate of excretion (5, 6). An appreciable percentage also appears in the pancreatic juice, the level rising and falling together with fluctuations in the enzymatic activity of the juice (7).

[1] This work was supported by the United States Atomic Energy Commission.

[2] Present address: Department of Anatomy, The Johns Hopkins University School of Medicine, Baltimore 5, Maryland.

[3] This investigation was conducted during the tenure of a Damon Runyon Memorial Fund Research Fellowship. Present address: GI Section, the Hospital of the University of Pennsylvania, Philadelphia, Pa.

[4] Abbreviation for the di-sodium salt of ethylenediaminetetraacetic acid.

METHODS

Mn^{56} ($t_{1/2}$: 2.59 hrs.), and Mn^{52} ($t_{1/2}$: 5.8 days) were used as tracers in these studies, the former being prepared in the Brookhaven nuclear reactor, and the latter in the Brookhaven cyclotron. Both isotopes were checked for radiological purity by following the rate of radioactive decay for at least five half-lives, with no significant deviation from the expected rates noted. The subjects received doses of 0.1 to 1.0 mc., containing less than 1.0 mg. of Mn^{++}, administered directly into the tubing of a rapidly running intravenous infusion of 5 per cent glucose in water, and washed in with about 100 ml. of the solution. In two experiments, the dose was mixed with a 10 to 50-fold excess of EDTA (buffered at pH 7.4) prior to administration. Standards for comparison were made at the same time, by preparing suitable dilutions of the injected material. Experimental data are expressed as percentages of the dose administered.

Nine patients were studied, all of whom were considered to have malignant disease, but only one of whom (S. G.) was not ambulatory, and had clinical or laboratory evidence of impaired hepatic function. In none of the patients was there evidence of renal or gastrointestinal dysfunction.

The patient, S. G., had a biliary fistula through which approximately 40 per cent of an estimated 600-ml. total daily output of hepatic bile was passed. She had laboratory evidence of severely impaired hepatic function (BSP retention 100 per cent after 2 hrs.), and at autopsy three weeks later was found to have severe biliary cirrhosis.

Samples of heparinized whole blood were drawn at frequent intervals from all patients for up to four hours following injection. In three patients, 24-hour collections of urine and feces were carried out for six days. All of these patients had spontaneous, daily evacuations. In the patient (S. G.) with the external biliary fistula, three trials were carried out, and bile and urine were collected for 24-hour periods.

Counting of specimens and standards was done in a well-type scintillation counter. Samples of blood and urine were counted directly; weighed samples of feces were first homogenized in a Waring blendor, and weighed aliquots were counted. In two patients in whom retention of a large proportion of the dose was suspected, a body

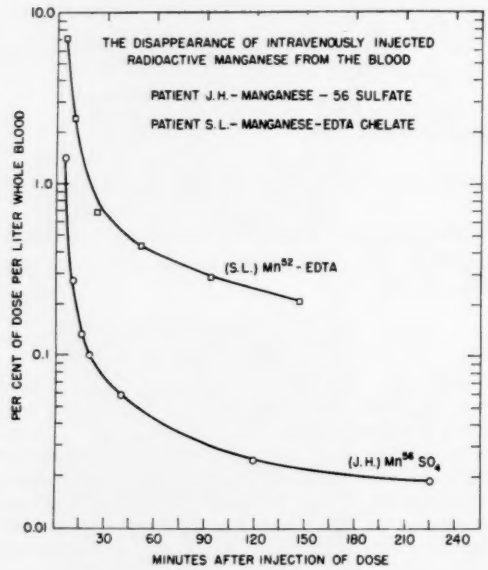

FIGURE 1

survey for radioactivity was made seven days after the injection of the Mn^{52}, using a radiation monitor.

Supplementary experiments were carried out with three groups of four white mice each. Each mouse received 0.25 ml. of buffered (pH 7.4) Mn^{52} solution intraperitoneally, containing a total of about 500,000 counts per minute. In addition, the three groups received carrier Mn or EDTA as follows: group 1 (low carrier) 1.0 mcg. Mn^{++}; group 2 (high carrier) 10.0 mcg. Mn^{++}; group 3 (chelate) 1.0 mcg. Mn^{++} and a ten-fold excess of EDTA. All mice were about 30 gm. in weight. At various intervals following injection, the mice were tightly confined in stoppered, ventilated 50-ml. lusteroid tubes. The whole mouse was then counted *in vivo* in a well-type G-M gamma counter, designed for counting large sample volumes. By comparison of the count at any given interval (count at t) with that obtained immediately after the administration of the dose (*t-zero* count), making corrections for radioactive decay, the per cent of the dose retained by the mouse at time t could be readily determined. A minimum of twelve measurements was made on each group over a total period ranging from 189 to 240 hours. Seventy per cent to 90 per cent of the dose was excreted during this interval.

This technique does not differentiate between urinary and fecal excretion, but by keeping the counting geometry constant—*i.e.*, carefully positioning and immobilizing the mice—it affords a simple and accurate method of measuring the rate of overall elimination. The results were plotted on semi-logarithmic paper in terms of per cent of dose retained against time, and subjected to statistical analysis.

RESULTS

Blood levels (Figure 1)

Ten similar blood curves were obtained in seven patients, which showed rapid decline of the manganese isotope level of the blood in the period following the intravenous injection of the material as $MnSO_4$ in aqueous solution. Two altered curves which, however, resembled each other, were obtained from two subjects following the administration of the Mn-EDTA chelate. Curves from representative patients are shown in Figure 1.

It can be seen that the "ionic" manganese (Mn in aqueous solution) curve fell more precipitously than did the Mn-EDTA curve, and remained at a lower level throughout the period of observation.

Urinary and fecal excretion (see Table I)

As shown in Table I, the urinary excretion of administered "ionic" manganese was almost negligible, but increased markedly when given as the Mn-EDTA chelate. The change induced by the chelating agent was immediate but of short duration, with no effect discernible after the second day. The fecal excretion of "ionic" manganese was appreciable, reaching its peak in the second or third day following administration. At the end of the six-day collection period, the radiation survey showed considerable remaining radioactivity, largely in the hepatic area.

Biliary excretion

In the patient with the biliary fistula a very small percentage of the injected Mn (average of 3 trials = 0.09 per cent) was recovered in the bile.

Table I
The Urinary and Fecal Excretion of Radiomanganese in Man

Patient	Amounts Expressed as Percent of Injected Dose					
	Collection Period, Days	Tracer Material Injected	% of Dose in Urine, First Day	Total Urinary Excretion	% of Dose in Feces, First Two Days	Total Fecal Excretion
R. B.	6	Mn^{52} in Water	0.45	0.74	20.5	31.4
M. D.	6	Mn^{52} in Water	0.21	0.23	3.15	8.4
S. G.	1	Mn^{56} in Water	0.13	0.13	—	—
S. G.	1	Mn^{56} in EDTA	4.56	4.56	—	—
S. L.	6	Mn^{52} in EDTA	16.6	16.7	0.8	6.7

EDTA is Ethylenediamine-tetraacetic Acid

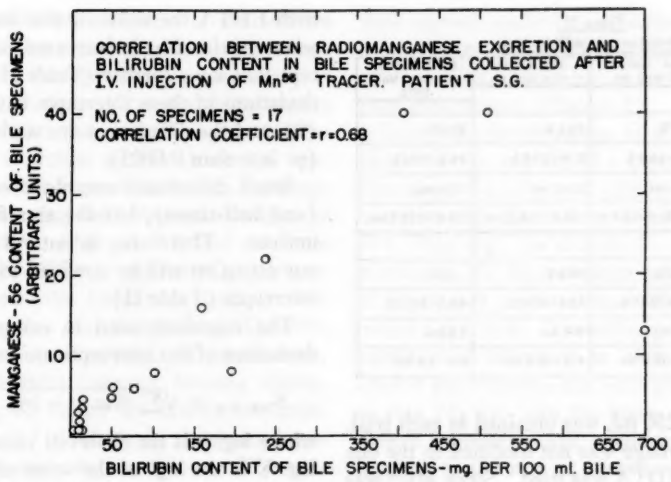

FIGURE 2

Retention of $Mn^{52}SO_4$ by Mice vs. Time. 1.0 mcg Mn^{++} in Dose (Low Carrier Group). Four Mice in Group. In Vivo Count Method

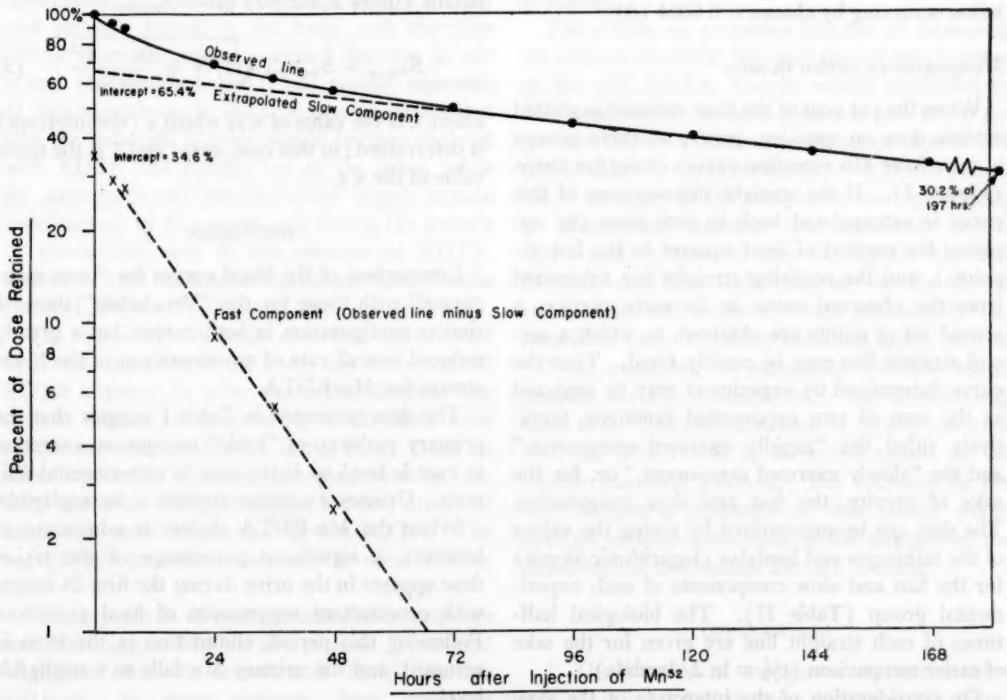

FIGURE 3

Table II

Retention of Mn^{52} by Mice

Group of Mice	Low Carrier 1.0 mcg. Mn	High Carrier 10.0 mcg. Mn	EDTA Chelate 1.0 mcg. Mn & EDTA
SLOW COMPONENT			
Intercept (a)	65.4%	25.2%	50.0%
1% Confidence Limits of a	61.1–69.9%	21.4–29.5%	45.5–55.1%
Half–Time ($t_{1/2}$)	168.1 hrs.	130.2 hrs.	204.1 hrs.
1% Confidence Limits of $t_{1/2}$	150.3–190.6 hrs.	108.3–163.2 hrs.	170.9–253.3 hrs.
FAST COMPONENT			
Intercept (a)	34.6%	74.8%	50.0%
1% Confidence Limits of a	32.4–37.0%	63.8–87.9%	44.6–54.0%
Half–Time ($t_{1/2}$)	13.0 hrs.	9.4 hrs.	7.6 hrs.
1% Confidence Limits of $t_{1/2}$	12.1–14.0 hrs.	6.3–18.9 hrs.	6.2–9.9 hrs.

although 200 to 250 ml. was obtained in each trial. This small percentage was not modified in the one trial where Mn-EDTA was used. Since Mn^{56} was used, biliary levels could not be followed for more than 24 hours. It was noted, however, that the great individual variations in Mn^{56} levels and bilirubin content appeared to coincide (Figure 2). Calculation of the coefficient of correlation gives a value for r: 0.68. The probability of such a correlation occurring by chance is 0.0024 (8).

Manganese excretion in mice

When the per cent of the dose retained is plotted against time on semi-log. paper, all three groups of mice have Mn retention curves of similar shape (Figure 3). If the straight line segment of this curve is extrapolated back to zero time (by applying the method of least squares to the last six points), and the resulting straight line subtracted from the observed curve in its early portion, a second set of points are obtained, to which a second straight line may be readily fitted. Thus the curve determined by experiment may be analyzed as the sum of two exponential functions, tentatively titled the "rapidly excreted component," and the "slowly excreted component," or, for the sake of brevity, the fast and slow components. The data can be summarized by giving the values of the intercepts and lambdas (logarithmic slopes) for the fast and slow components of each experimental group (Table II). The biological half-times of each straight line are given for the sake of easier comparison ($t_{1/2} = \ln 2/\text{lambda}$).

On consideration of the intercepts of the slow components, one can see that when Mn is chelated with EDTA the value of this intercept falls; and when carrier Mn is increased ten-fold, the intercept falls even further (Table II). The standard deviations of these intercepts have been calculated (9), and the differences are statistically significant (p: less than 0.0001).

Small differences are observed in the lambdas (and half-times), but the significance of these is unclear. Therefore, in interpreting these data, our attention will be confined to the difference in intercepts (Table II).

The equations used to compute the standard deviations of the intercepts are as follows:

$$S_{\log.\, y\cdot x} = \sqrt{\sum (\log. y - \log. Y)^2/N - 2}, \quad (1)$$

where log. y is the observed value of the log. of y, log. Y is the log. of the value of y obtained from the fitted line, and N is the number of points used in fitting the line.

$$S_{\log.\, b} = S_{\log.\, y\cdot x}/\sqrt{NS_x^2}, \quad (2)$$

where S_x is the standard deviation of the x's. Conversion of $S_{\log.\, b}$ from common to natural logarithms (times 2.302585) gives S_{lambda}.

$$S_{\log.\, a} = S_{\log.\, y\cdot x}\sqrt{1/N + \frac{(x - \bar{x})^2}{NS_x^2}}, \quad (3)$$

where x is the value of x at which a (the intercept) is determined; in this case, zero; and $\bar{x}$ is the mean value of the x's.

DISCUSSION

Comparison of the blood curves for "ionic manganese" with those for the "Mn-chelate" shows a similar configuration in both curves, but a greatly reduced overall rate of movement out of the blood stream for Mn-EDTA.

The data presented in Table I suggest that the primary pathway of "ionic" manganese excretion in man is fecal, as is the case in experimental animals. Urinary excretion appears to be negligible.

When the Mn-EDTA chelate is administered, however, a significant percentage of the tracer dose appears in the urine during the first 24 hours, with concomitant suppression of fecal excretion. Following this period, elimination in the feces is resumed, and the urinary Mn falls to a negligible level.

The biliary excretion data, although inadequate

to give any quantitative estimates of the relative importance of this route in man, does offer a suggestive correlation between the Mn and bilirubin content of the specimens. The possibility that a Mn-bilirubin complex may indeed play an important role in the excretion of this element is supported by some data obtained by the ion exchange technique for measuring the formation constants of complex ions (10). At pH 7.4 and an ionic strength of 0.16 M (Na+)—approximately physiological conditions—the formation constant of a Mn-bilirubin complex was found to be K_f: 1700. In contrast, a representative bile acid (cholic acid) showed no measurable complex forming ability. For comparison, K_f values for Mn-citrate: 2900, and Mn-EDTA: 1.7×10^7 were determined under the same experimental conditions. The adenosine polyphosphates, plasma proteins, and some other physiologically active substances also proved to be able to complex manganese (1, 11, 12).

The experimental data suggest that, after injection, Mn follows at least two metabolic pathways. These findings can be explained by the assumption that one portion of the dose is quickly and strongly bound in the body, and therefore slowly excreted, while a second fraction is unbound (or weakly bound), and rapidly excreted. If this hypothesis is valid, it can be anticipated that the unbound fraction would become chelated with EDTA and rapidly lost in the urine, while the strongly bound fraction would largely remain uninfluenced by this agent, and follow the pattern of elimination seen in the absence of EDTA. Review of both the human and mouse data (Tables I and II; Figure 3) supports this assumption.

The hypothetical kinetics described above could be further modified in the body, and the effects of EDTA lessened, by other competing divalent ions such as calcium or magnesium, which could form complexes with the EDTA in the dose. Such an effect is probably minimal, since the formation constant of the Ca-EDTA complex is many orders of magnitude smaller than that for Mn-EDTA. The Mg-EDTA formation constant is even smaller (1).

This hypothesis of multiple metabolic pathways was first suggested by the two patients (R. B. and M. D.) in whom the excretion of "ionic" Mn was followed. In these subjects, fecal excretion reached a peak on the second day (Table I), and then slowed markedly. Confirming this suggestion, the mouse studies showed Mn retention curves which can be readily analyzed into fast and slow components (Figure 3).

Likewise, consideration of these same two patients (R. B. and M. D.) permits some speculation about the effects of additional carrier on Mn excretion. R. B. received about 40 times as much Mn (0.66 mg.) as did M. D. (0.017 mg.), and during the six days of observation R. B. excreted 31.4 per cent of the dose, while M. D. excreted only 8.4 per cent. It would appear that the rate of excretion is increased as more carrier is given. Such a phenomenon has been previously observed in rats (5, 6). On the basis of the multi-component hypothesis, one would anticipate that this increase in the rate of excretion would be due to an increase in the proportion of the total dose being eliminated via the unbound, rapidly excreted components. From the mouse data, one finds (Table II) that a ten-fold increase in carrier raises the intercept of the rapidly excreted component from 34.6 per cent to 74.8 per cent, as predicted.

The effects on excretion kinetics of increasing the amount of carrier Mn, and that of pre-chelation of Mn with EDTA, may be readily explained by this hypothesis. In both instances the effect appears to be one of increasing the proportion of Mn excreted in the rapid component. It seems plausible to assume that the manganese binding capacity is limited, and therefore that additional carrier Mn would be shunted into the rapidly excreted fraction, and have little influence on the slowly excreted, bound fraction, which presumably has been saturated. A strong chelating agent, such as EDTA, would act by competing with the bound fraction for the available Mn, and sequestering some of it. Since EDTA itself is rapidly excreted (1), the Mn bound by it would appear in the rapidly excreted fraction. The increase in the fast component produced by this means would have an inverse relationship to the strength of the physiological mechanism for Mn binding.

CONCLUSIONS

1. The kinetics of manganese excretion were studied in man and in mice following the intravenous injection of a radioactive tracer.

2. Rapid removal of manganese from the circulating blood occurred, and slowing of this removal after pre-chelation of the dose with EDTA was observed.

3. Manganese excretion in the patients studied was primarily fecal. When pre-chelated with EDTA, Mn is eliminated almost exclusively in the urine for about 24 hours, and then fecal excretion is resumed.

4. Excretion curves obtained from mice could be analyzed graphically into fast and slow components. Increasing carrier Mn, and pre-chelation of Mn with EDTA both increased significantly the intercept of the fast component.

5. It is suggested that the degree of influence on the excretory pattern of manganese exerted by pre-chelation with EDTA is an inverse function of the degree and type of physiological binding of this element.

6. It is suggested that the observed behavior of manganese is best explained by assuming the existence of bound and unbound fractions in the body.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Dr. George C. Cotzias, Medical Department, Brookhaven National Laboratory, whose comments and suggestions were of great assistance in interpreting our data; and to Dr. Margaret Merrell, Department of Biostatistics, Johns Hopkins University School of Hygiene and Public Health, who advised us on the statistical analysis of our data.

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THE CHEMICAL ESTIMATION OF EPINEPHRINE AND NOREPINEPHRINE IN HUMAN AND CANINE PLASMA. I. A CRITIQUE OF THE ETHYLENEDIAMINE CONDENSATION METHOD[1]

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(Submitted for publication February 21, 1956; accepted March 15, 1956)

The recovery of epinephrine (E) and norepinephrine (N) from plasma on activated alumina is reproducible and this adsorption technique can be made reasonably specific for catechols. The development of chemical methods for the assay of E and N in peripheral plasma has been hampered, however, by: 1) the low concentrations of E and N normally present (1 μg. per L. or less); 2) the lack of specificity of the methods for E and N in the presence of other catechols which are excreted in urine (1, 2) and thus presumably are present in blood; and 3) difficulty in obtaining "blanks" from plasma extracts or eluates, a condition usually met in methods designed to measure endogenous substances.

In 1949 and 1950, Lund (3–5) described a method based on the conversion of E and N to their fluorescent trihydroxyindole derivatives. Only dihydroxyphenyl compounds possessing a side chain which contains both an hydroxyl and an amino group appear to be detected by this method, and "blanks" can be obtained by allowing oxidation of the trihydroxyindole derivatives of E and N to proceed to a non-fluorescent state. However, the sensitivity of the method appears insufficient to permit measurements of E and N in small quantities of normal peripheral plasma (5). Simultaneously, Natelson, Lugovoy, and Pincus (6) described the condensation of E with ethylenediamine (EDA) to form a highly fluorescent product. In 1952 and 1953, Weil-Malherbe and Bone (7, 8) extended the use of this procedure to the simultaneous estimation of E and N in blood by measuring the intensity of fluorescence at two different wave lengths. Modifications (9, 10) have been reported, and the method appears to be in frequent use. It is less specific than the Lund (trihydroxyindole) method, and will detect the presence of dihydroxyphenyl compounds other than E and N (7).

The levels of E and N found in normal human plasma by Weil-Malherbe and Bone (1.3 and 5.2 micrograms per liter, respectively) differ from those observed by others (10–12) using essentially the same method, and from the levels obtained by bioassay (13). In view of the fact that a reliable method for estimation of E and N can assume importance in the interpretation of physiological changes in man, it was considered necessary to re-evaluate the EDA method and to compare the results obtained with this technique with those obtained by the trihydroxyindole method. Our results with both dog and human plasma indicate that the EDA method is not specific for N, and may not be so for E.

METHODS

Blood was collected in heparinized syringes, the cells separated by centrifugation, and to a measured amount of the plasma one-half its volume of sodium fluoride (2 per cent)-sodium thiosulfate (3 per cent) solution was added.

Removal of E and N from plasma was accomplished by adsorption of the catechol amines on activated alumina at pH 8.0 to 8.5, followed by elution from the alumina column with acetic acid as described by Weil-Malherbe and Bone (7). Woelm alumina, non-alkaline, Grade I (Alupharm Chemical Co., Elmont, L. I.) was found uniformly low in blank value and satisfactory for routine use. Pressure was used instead of suction on the columns in order to avoid vaporization of the solvent within the column bed.

A Farrand fluorometer with output connected to a Rubicon galvanometer was used to measure fluorescence. The primary filter used for the EDA method was an interference filter with a transmission peak at 436 mμ plus a Wratten gelatin filter No. 35; secondary filters were 1) a green filter pair composed of an interference filter

[1] Supported in part by Grant H-1568(C) from the United States Public Health Service.

with a peak at 500 mμ plus a Wratten gelatin filter No. 57, and 2) an interference filter with a peak at 580 mμ.

The permanent standard used was a 10-ml. isobutyl alcohol extract of 10 ml. of water containing 20 micrograms of E which had been condensed with EDA; this was stored at 4°C and diluted 1:100 with isobutyl alcohol for use as needed to maintain instrument sensitivity at a preassigned value (90.0 divisions at diaphragm opening No. 3) for each secondary filter.

I. Ethylenediamine method. Two major modifications were necessary before the Weil-Malherbe and Bone method could be used. They were required because: 1) the fluorescence of E condensate in alumina eluate is roughly 150 per cent of its value in water or acid solution, and 2) the fluorescence of N condensate decays exponentially and irreversibly in strong blue or green light. Modifications were made as follows: 1) preparation, for use with each analysis of two standards of E and N (0.04 microgram of base per 10 ml.) and of two reagent blanks, each in 5 ml. of 0.33 M acetic acid which has been passed through a 0.7 gram column of alumina previously prepared in water, plus water to make a total volume of 10 ml.; 2) following the addition of EDA, all samples were exposed to light (dim room light only) for the same length of time. Readings were made through the secondary green filter pair first, noting the galvonometer deflection at a constant time interval (five seconds) after opening the shutter; the procedure was then repeated using the yellow secondary filter. This allowed for completion of galvonometer response, allowed equal decay-time of N condensate for all samples, and reduced the fluorescence by the least amount possible. (The blue-green fluorescence of ethylenediamine condensates of noradrenaline and certain other catechols decays rapidly when these substances are excited by light of 436 mμ; the yellow emission is relatively stable.)

II. Trihydroxyindole method. The method of Lund (5) as modified by von Euler and Floding (14) was used essentially as described to assay E and N. Blanks were prepared by adding ascorbic acid to the plasma eluate before the ferricyanide, thus preventing conversion of E and N to their respective chromes. The intensities of fluorescence due to E and N were determined, using two primary interference filters (400 and 438 mμ).

In both methods calculations of E and N levels were made by solving simultaneous equations involving the net galvanometer deflections (fluorescence above blank) observed using the two filters.

RESULTS

I. Specificity

Specificity of the EDA method was studied in three ways: a) by the addition of certain mono- and di-hydroxyphenyl compounds [2] to plasma, followed by estimation of their recovery by alumina column chromatography, b) by condensation of the substances with EDA and comparison of their fluorescence spectra with those of E and N condensates, and c) by comparison of apparent E and N levels in the same plasma eluates by both the EDA and trihydroxyindole methods. The compounds selected were some of those which might be expected to be present in blood, and which could interfere in the EDA method.

A. Recovery. The recoveries of dihydroxyphenylalanine (dopa), 3-OH tyramine (dopamine,) 3,4-dihydroxyphenyl acetic acid ("dopac"), isopropylnoradrenaline, and 5-hydroxytryptamine (serotonin), after addition to plasma and assay by the EDA method, are shown in Table I, together with representative recoveries of E and N. Serotonin was the only compound tested which was recovered in negligible amounts, due to non-adsorption on alumina. Omission of the fluoride-thiosulfate preservative from the plasma did not reduce the recovery of E, reduced that of N by less than twenty per cent, but diminished that of dopac by more than fifty per cent.

B. Relative fluorescence of condensation products. Standard solutions of the mono- and dihydroxyphenyl compounds were made up in the same manner as the usual E and N standards, using EDA condensation. The fluorescence intensities of these compounds and of E and N are given in Table II. The intensity of fluorescence of added E obtained with the secondary yellow filter

TABLE I

Recovery of hydroxyphenyl compounds added to plasma (EDA method)

Compound	Micrograms added	Per cent recovered
1-Epinephrine (3,4-dihydroxyphenyl-B-methyl aminoethanol)	0.001–0.20	78
1-Norepinephrine (3,4-dihydroxyphenyl-B-aminoethanol)	0.005–4.0	80
Dopac (3,4-dihydroxyphenyl acetic acid)	0.08–0.40	65
Dopamine (3,4-dihydroxyphenyl-B-aminoethane)	0.02–50	79
Dopa (3,4-dihydroxyphenyl-B-aminopropanoic acid)	50	94
Isopropylnoradrenaline (3,4-dihydroxyphenyl-B-isopropylaminoethanol)	0.25–0.50	72
Serotonin (5-hydroxyindole acetic acid)	150–250	0.17

[2] We wish to thank Dr. Richard Dahlbom, of Astra, Ltd., for kindly supplying 3,4-dihydroxyphenyl acetic acid, and Dr. M. L. Tainter, Sterling-Winthrop Research Institute, for l-epinephrine and l-norepinephrine.

has been arbitrarily assigned a value of 100 (actual value = 930 divisions per microgram).

C. Comparison of EDA and trihydroxyindole methods. Approximately 1.5 L. of arterial plasma was obtained by exsanguinating three dogs under light pentobarbital anesthesia (25 mg. per Kg. intravenously). The catechols were eluted from twenty 100-ml. lots of plasma-preservative mixture on two gram alumina columns, washed with sodium acetate solution (5 ml. of 0.2 M) and water (5 ml.), and eluted in 5 to 10 ml. of 0.3 M acetic acid. Aliquots of the combined eluates from each dog's plasma were analyzed using both the EDA and trihydroxyindole methods. Blanks and additions of E and N were prepared from each eluate for assay by the trihydroxyindole method. There was good agreement between the E levels estimated by the two methods (E = 1.78 μg. per L. by EDA and 1.84 μg. per L. by trihydroxyindole); however, N levels were invariably less by the trihydroxyindole method and averaged only 30 per cent of the level obtained by the EDA method (N = 0.85 by EDA and 0.29 by trihydroxyindole).

Smaller quantities of human plasma (25 to 50 ml.) were separated from blood drawn from the antecubital veins of four normal volunteers, and assayed for E and N by the trihydroxyindole method. The mean values were (μg. per L.) E = 0.00 ± 0.005 (S.E.) and N = 0.20 ± 0.097 (S.E.). A 10-ml. sample of plasma was obtained by similar means from each of 17 normal subjects and the E and N levels estimated by the EDA method. The mean values were (μg. per L.) E = 0.097 ± 0.034 (S.E.) and N = 2.74 ± 0.277 (S.E.); both are significantly ($p < 0.01$ by Fisher t test) greater than the levels obtained using the trihydroxyindole method.

Three 30-ml. samples of normal human plasma were divided in half. Preservative was added to one half but not to the other, and both were analyzed by the EDA method. The apparent E level was increased by an average of 0.07 μg. per L. when preservative was omitted, while that of N was reduced to from thirty to fifty per cent of that estimated in the preservative-treated fraction.

TABLE II

Fluorescence of hydroxyphenyl compounds (EDA method)

Compound	Relative fluorescence intensity with filter No. 580 (yellow)	500 (green)	Fluorescence ratio (500/580)
Epinephrine	100	58	0.58
Norepinephrine	34	127	3.74
Dopac	5.1	21	4.04
Dopamine	30.9	35	1.13
Dopa	0.65	1.1	1.69
Isopropylnoradrenaline	59	43	0.73
Serotonin	2.2	4.9	2.22

II. Sensitivity

The sensitivity of the EDA method for E and N is limited largely by variation in the blanks. Addition and recovery experiments indicate that as little as 0.001 to 0.002 μg. E and 0.005 to 0.010 μg. N can be detected in plasma eluates.

DISCUSSION

To facilitate discussion the E and N levels (μg. per L.) found in human peripheral plasma by various workers have been summarized in Table III. It is evident that the E levels estimated by most workers using the EDA method are in the same range (0.0 to 0.2 μg. per L.) as those estimated by bioassay or by the trihydroxyindole method, while the N levels measured by the latter are only a

TABLE III

Epinephrine and norepinephrine concentration in human peripheral plasma (μg. per L.)

Method	No. of subjects	E±S.D.	N±S.D.	Author
Guinea-pig uterus	1	<0.06	<1.0	Holzbauer and Vogt, 1954
Trihydroxyindole	4	0.00 ± 0.01	0.20 ± 0.19	Valk and Price, 1956*
EDA (with preservative)	17	0.097 ± 0.14	2.74 ± 1.14	Same
EDA (without preservative)	3	0.083 ± 0.16	1.26 ± 1.32	Same
EDA	7	0.14 ± 0.21	3.96 ± 1.7	Manger and co-workers, 1954
EDA	6	0.4 ± 0.2	2.1 ± 1.3	Aronow, 1956
EDA	22 males	1.18 ± 0.21	5.29 ± 1.10	Weil-Malherbe and Bone, 1953
	21 females	1.46 ± 0.38	5.16 ± 0.74	

* Uncorrected for losses in recovery (ten to twenty per cent).

tenth as great as by the EDA method. This is evidence for the presence in plasma of a substance which: 1) is adsorbed on alumina at pH 8.0 and eluted by dilute acid, 2) has a fluorescence ratio (net fluorescence read using green secondary filter divided by that read on yellow filter) similar to that of N by the EDA method, and 3) is not detected by the trihydroxyindole method. If the fluorescence ratio of the interfering substance differed markedly from that of N, solution of the simultaneous equations used to calculate E and N by the EDA method would yield a false value for E, and the apparent E levels calculated by the two methods would differ considerably. Comparison with the results obtained by Holzbauer and Vogt (13) further suggests that the substance interfering in the EDA method is either not recovered by their procedure or does not inhibit carbachol-induced contractions of the guinea pig uterus.

Among the catechol substances which have been reported present in human urine, tissue extracts, or blood are: 1) isopropylnoradrenaline, tentatively identified by Lockett (15) in the adrenal medulla; 2) dopamine, found in urine (1), and 3) dopac which has been tentatively identified in urine by von Euler, von Euler, and Floding (2). Dopa has not been shown to be present in tissue or biological fluids, nor has dihydroxyphenylserine, and isopropylnoradrenaline constitutes less than one per cent of the E and N in the human adrenal medulla (15). Therefore, the principal possibilities for the substances detected in plasma eluates by the EDA method (but not by the trihydroxyindole method) appear to be dopamine and its supposed metabolite dopac. Both substances are adsorbed from plasma by alumina and are eluted by dilute acetic acid; also, neither substance produces significant fluorescence by the trihydroxyindole method. However, the EDA condensate of dopamine has a fluorescence ratio different from that of either E or N, while the fluorescence ratio of dopac condensate differs from that of N by less than ten per cent. Furthermore, dopac is apparently biologically inactive (2). A substance present in plasma and interfering in the EDA method may therefore be dopac.

Further evidence for the presence of a dopac-like substance in plasma was obtained by omitting the addition of preservative to same plasmas, thereby reducing the recovery of authentic noradrenaline from the plasma only slightly while diminishing that of dopac to approximately thirty per cent. This technique resulted in much better agreement between the "N" levels estimated by the EDA and trihydroxyindole methods than occurred when preservative was used.

Since the fluorescence ratios of N and dopac are slightly different the presence of dopac in plasma eluates will introduce a systematic error in the estimation of E by formulae involving the fluorescence ratio observed for N. Recalculation of the data obtained from normal human plasma indicates that the average E level of 0.1 micrograms per liter should be more nearly 0.2 to 0.3 micrograms per liter, assuming at least two-thirds of the "N" value to be due to the presence of dopac. This value thus exceeds the level calculated by the trihydroxyindole method by at least 0.2 micrograms per liter. This difference could have a number of explanations, among which are 1) the presence of a fourth catechol in plasma, or 2) a systematic error in either method.

The levels of "E" and "N" obtained by Aronow and Howard (10, 11) and Manger and his coworkers (12) using the EDA methods are in substantial agreement with our own, but differ greatly from those estimated by Weil-Malherbe and Bone (8). Further, we have been unable to confirm the finding of Weil-Malherbe and Bone that there is an association of "E" and "N" with platelets (16) and we have observed an increase in plasma "E" during insulin hypoglycemia in man (17) rather than a decrease which they (18) have reported. It may be concluded that differences in method other than those described must exist.

The sensitivity and precision of the EDA methods appear sufficient to permit detection of quantities of E and N in plasma of the order of 10^{-3} μg. However, the specificity of the methods for N in plasma eluate is inadequate, while specificity for E is not established. If further effort is to be expended on methods to determine E and N levels in peripheral plasma by chemical means, it appears likely that procedures more specific than EDA condensation must be employed.

SUMMARY AND CONCLUSIONS

1. The ethylenediamine condensation method is capable of detecting amounts of epinephrine and

norepinephrine of the order of 10^{-3} micrograms after their addition to plasma.

2. The method is not suitable for estimating the norepinephrine concentration in either canine or human peripheral plasma.

3. Changes in epinephrine concentration can be measured provided that the concentrations of other catechols in plasma remain unchanged.

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CLINICAL DETERMINATION OF THE SITES OF RED CELL SEQUESTRATION IN HEMOLYTIC ANEMIAS[1]

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(Submitted for publication January 30, 1956; accepted April 3, 1956)

The widespread application of red cell survival techniques has revealed the importance of excessive red cell destruction in the pathologic physiology of many of the anemias. An increasing array of *in vitro* methods for detecting red cell or serum abnormalities has provided insight into the *in vivo* mechanisms underlying some of these processes. In certain disease states the presence of visible or physically measurable alterations of the red cells has permitted detection of the sites and to some extent of the mechanisms of sequestration of these cells. Such valuable observations have been made upon pathologic material from patients with congenital hemolytic anemia (1–5) and sickle cell anemia (1, 5–7). The need has continued to exist, however, for a clinical method by which to determine the sites of red cell destruction, both for an understanding of the relationship between red cell abnormalities and the body's red cell clearing mechanisms, and for pragmatic aid in the often difficult decisions concerning splenectomy.

The potential usefulness of $Na_2Cr^{51}O_4$-labelled red cells in the clinical determination of the sites of red cell destruction was suggested to us by observations on postmortem tissues taken from a patient with the hemolytic anemia of liver disease, who succumbed during observation of the survival of Cr^{51}-labelled autogenous red cells.[2] The Cr^{51} activity of the patient's splenic tissue was greater than that of other tissues even when correction was made for the Cr^{51} activity of the residual red cells. Moreover, the radioactivity of the packed red cells removed from the spleen exceeded that of a comparable sample of packed red cells from the peripheral blood. In order to investigate the possibility that Cr^{51}-labelled red cell deposition could be determined by measuring body surface radioactivity, several questions required exploration: 1) Are the emanations of Cr^{51} suitable for external body scanning at safe dosage levels? 2) Does the site of tissue deposition of Cr^{51} following the intravenous injection of Cr^{51}-labelled red cells necessarily indicate the site of deposition of whole red cells? 3) Is the turnover of Cr^{51} deposited in tissues sufficiently slow to provide a detectable accumulation? 4) Finally, do the results obtained confirm the information already established in certain specific instances, for example, in congenital hemolytic anemia and in sickle cell anemia? In order to investigate these questions, observations were first made on the distribution of Cr^{51} in normal subjects. The fate of Cr^{51}-labelled autogenous red cells was then observed in patients with known intracorpuscular defects and, lastly, in patients with certain diseases generally characterized by excessive red cell destruction and a preliminary report (10) was made.

METHODS

Cr^{51} labelling technique. All red cell labelling was performed under sterile conditions with $Na_2Cr^{51}O_4$[3] diluted with normal saline to a concentration of about 30 micrograms of chromium per cubic centimeter. Between 100 and 150 microcuries of Cr^{51} in this form were added with immediate mixing to 50 cc. of whole blood in a siliconized flask containing 12 cc. of acid-citrate-dextrose solution. The flask was then gently agitated continuously for 45

[1] This investigation was supported in part by a grant from the Helen Hay Whitney Foundation and by Research Grant RG3507(C3) from the National Institutes of Health, Public Health Service.

[2] Cr^{51} either as $Cr^{51}O_4^{--}$ (8) or as Cr^{51+++} (9) is capable under appropriate conditions of firmly labelling red cells by chemical attachment to the hemoglobin and to the red cell membrane, respectively. For brevity, however, red cells labelled with $Na_2Cr^{51}O_4$ will be described here as "Cr^{51}-labelled red cells."

[3] "Rachromate," Abbott Laboratories, North Chicago, Illinois.

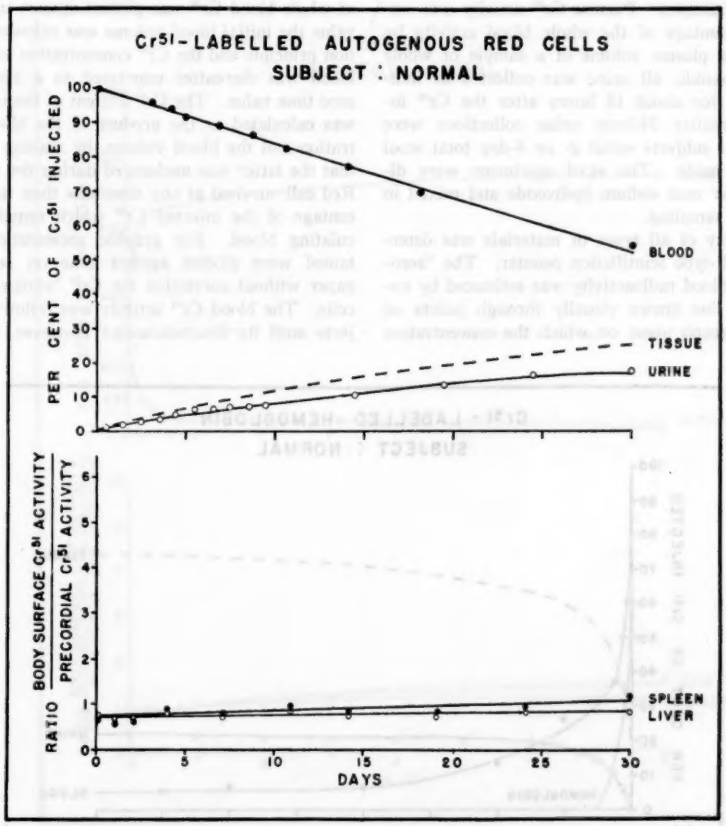

FIGURE 1

The gradual, almost linear, disappearance of Cr^{51}-labelled normal autogenous red cells from the blood stream shown in the upper portion of the figure is reflected by a gradual accumulation of Cr^{51} in the tissues. Little difference exists in the levels of relative radioactivity over liver and spleen, as depicted in the lower portion of the figure.

minutes. Unbound or reduced chromium was removed by washing the red cells once in sterile normal saline, after which the red cells were suspended in 2 volumes of normal saline preparatory to injection. Over 99 per cent of the Cr^{51} injected was in the red cells.

Solutions of Cr^{51}-labelled hemoglobin were prepared by similarly labelling red cells which were thereafter washed 5 times in normal saline and then hemolyzed with 4 volumes of distilled water.[4] After adjusting the sodium chloride concentration of the suspending fluid to 0.85 gm. per cent, the red cell "ghosts" were centrifuged down and the supernatant solution was Seitz-filtered prior to injection.

A solution of a reduction product of $Na_2Cr^{51}O_4$, $Cr^{51}Cl_3$,[5] was diluted with normal saline to a suitable volume; this was injected directly intravenously in one instance and after preliminary incubation with serum in another.

Specimen collection and Cr^{51} determination. Following injection of Cr^{51}-containing preparations, venous blood specimens were collected at appropriate intervals in bottles containing dry "balanced" oxalate. Their radioactivity was determined after hemolysis by freezing and thawing. Specimens for the measurement of plasma radioactivity were drawn into saline-wetted syringes and the citrated plasma removed after centrifugation of the specimens within one hour of their procurement. The plasma samples were then frozen and thawed along with

[4] It is equally feasible to label the hemoglobin in solution after hemolysis; this may be done either with $Na_2Cr^{51}O_4$ or $Cr^{51}Cl_3$.

[5] Radioactive chromic chloride, Abbott Laboratories, North Chicago, Illinois.

the whole blood samples. Plasma Cr^{51} activity was expressed as a percentage of the whole blood activity by correction for the plasma volume of a sample of whole blood. When possible, all urine was collected as individual specimens for about 18 hours after the Cr^{51} injection, and thereafter 24-hour urine collections were made. In several subjects serial 2- or 4-day total stool collections were made. The stool specimens were digested with 10 per cent sodium hydroxide and mixed in a blender before sampling.

The radioactivity of all types of materials was determined with a well-type scintillation counter. The "zero-time" peripheral blood radioactivity was estimated by extrapolation of a line drawn visually through points on semilogarithmic graph paper on which the concentration of whole blood Cr^{51} was plotted against time. From this value the initial blood volume was calculated by the dilution principle and the Cr^{51} concentration of the circulating blood was thereafter expressed as a percentage of the zero time value. The Cr^{51} content of the circulating blood was calculated as the product of the blood Cr^{51} concentration and the blood volume, by making the assumption that the latter was unchanged during the period of study. Red cell survival at any time was then taken as the percentage of the injected Cr^{51} which remained in the circulating blood. For graphic presentation data so obtained were plotted against time on rectilinear graph paper without correction for Cr^{51} "elution" from the red cells. The blood Cr^{51} activity was followed in most subjects until its disappearance. However, for easier com-

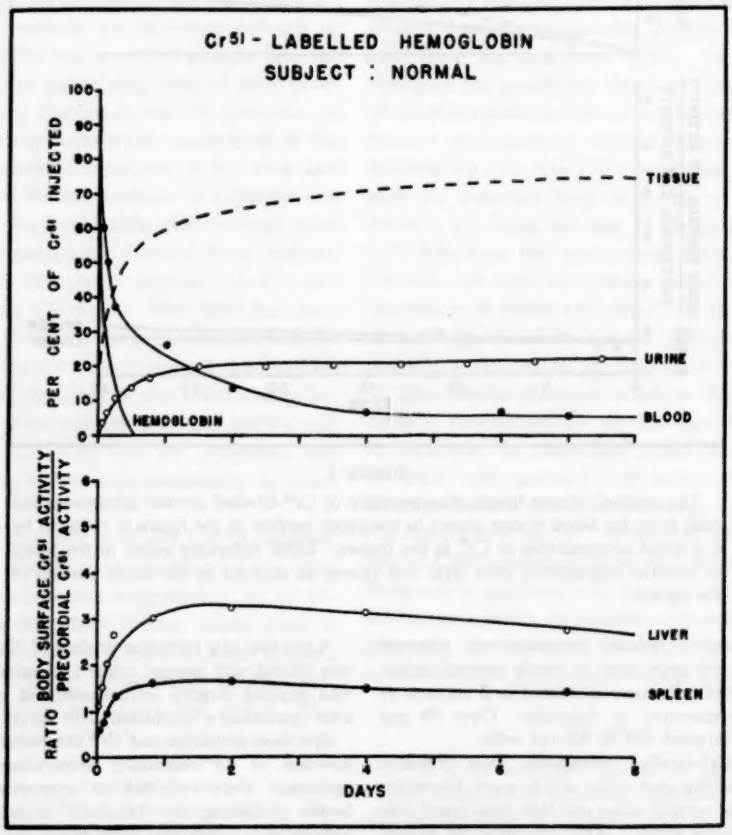

FIGURE 2

Following intravenous injection of Cr^{51}-labelled hemoglobin solution, radioactivity disappeared from the blood at a continuously slowing rate with a half-disappearance time of 3 hours, whereas by chemical determination plasma hemoglobin was removed from the circulation "exponentially" with a half-time of from 1½ to 2 hours. With the removal of Cr^{51} from the peripheral blood, there was a rapid, prominent rise in radioactivity over the liver and to a lesser extent over the spleen.

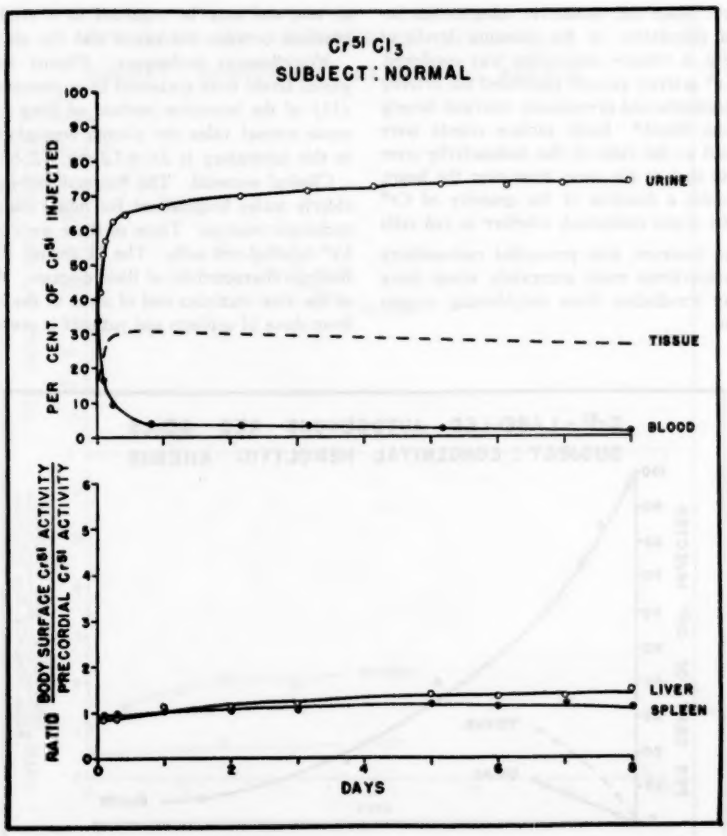

FIGURE 3

The upper portion of this figure indicates the great rapidity with which injected cationic trivalent Cr^{51} in saline is cleared from the circulation. Most of this chromium appeared in the urine, with only a slight accumulation by the liver and spleen. Preliminary incubation of $Cr^{51}Cl_3$ and serum before injection enhanced the relative Cr^{51} uptake by the liver.

parison of data, a time axis of only 30 days was employed in the presentation of the studies of individual patients shown below in Figures 1 to 13. The urinary excretion of Cr^{51} was expressed as the cumulative percentage of the injected dose of Cr^{51}. Since in no instance was Cr^{51} excreted in the patients' stools, the chromium not accounted for in blood or urine was designated as "tissue Cr^{51}" in the figures and in Table I.

Body surface counting. Body surface counting was performed with a directional scintillation counter having a conical collimator 4 inches in depth and 3 inches in internal diameter at the open end. Twenty minutes after the injection of materials labelled with Cr^{51}, each patient was scanned. In no instance among the patients reported here were high levels of activity found other than over the heart, liver, or spleen. In each patient, therefore, surface projections of these organs were determined by physical examination and their approximate centers were marked with a skin pencil. In some patients radioactivity levels were also followed over the thoracic, lumbar and sacral areas. In performing each count the scintillation counter was plumb-centered over the appropriate mark with the patient in a standardized position, permitting the body surface overlying the organ to approximate parallelism with the collimator surface. The counter was then lowered to direct apposition with the body surface and two or more measurements were made with an automatic scaler.

In measuring the body surface radioactivity, it was recognized that a variable but important degree of radioactivity over each organ derived from the activity of the perfusing blood, as well as from stray radiation from one

organ to another. Since the geometric complexities involved prevented calculation of the absolute levels of organ radioactivity, a relative expression was employed. The precordial Cr^{51} activity grossly paralleled the activity of whole blood specimens and presumably emerged largely from the perfusing blood.[6] Body surface counts were therefore expressed as the ratio of the radioactivity over a certain organ to that at the same time over the heart. This ratio represents a function of the quantity of Cr^{51} accumulated in the organ concerned, whether in red cells or not, and may be regarded as a crude measure of a gradient between the organ and the circulating blood.

Miscellaneous techniques. Plasma and urine hemoglobin levels were measured by a photoelectric adaptation (11) of the benzidine method of Bing and Baker. The mean normal value for plasma hemoglobin concentration in this laboratory is 2.6 ± 1.2 mg. (2 S.D.) per cent.

Clinical material. The "normal" subjects studied were elderly males hospitalized for minor illnesses and/or for sociologic reasons. Three of these were given autogenous Cr^{51}-labelled red cells. The 11 anemic patients displayed findings characteristic of their diseases. A brief summary of the vital statistics and of some of the hematologic data from these 14 subjects and patients is presented in Table I.

[6] It is apparent, however, that precordial radioactivity reflects blood radioactivity most accurately when there is the least stray irradiation from neighboring organs such as the spleen.

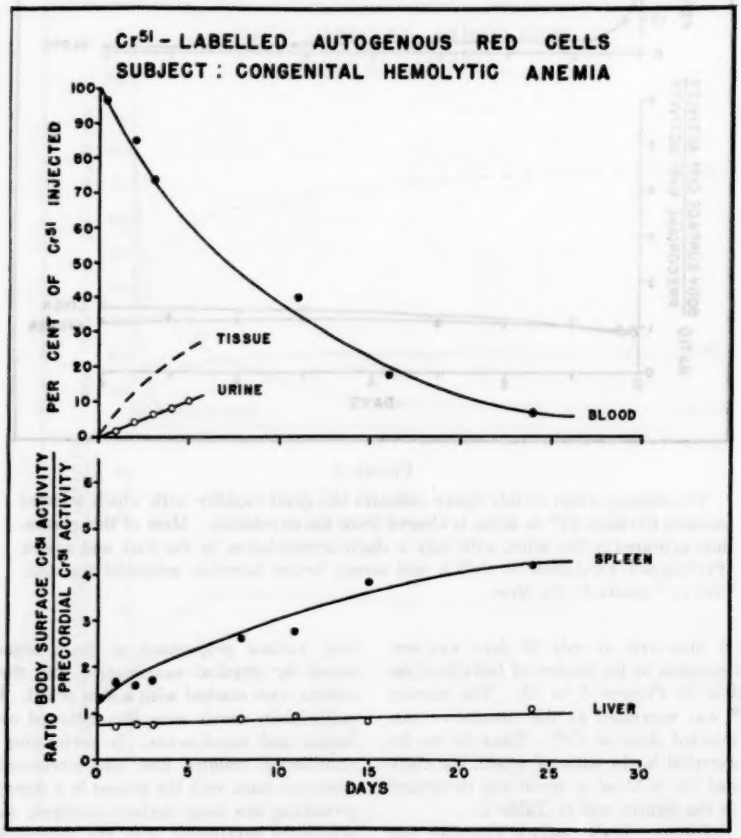

FIGURE 4A

The increased rate of Cr^{51}-labelled red cell destruction in a patient, Case 1, with moderately severe congenital hemolytic anemia (hereditary spherocytosis) was attended by a predominant uptake of radioactivity over the spleen. Note both the increased splenic activity at zero-time and the manner in which Cr^{51} is progressively deposited in the spleen in proportion to its removal from the blood stream.

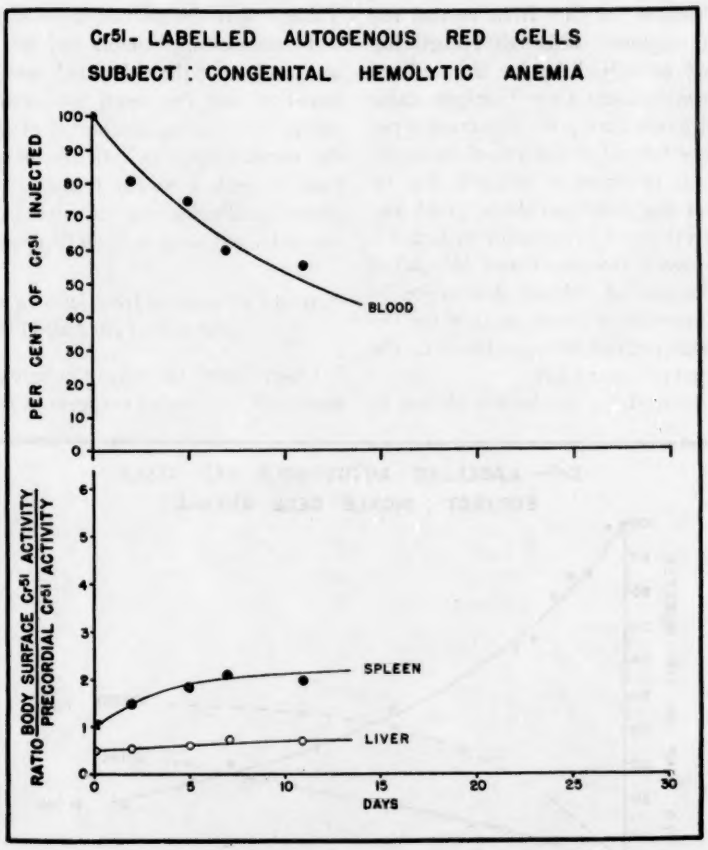

FIGURE 4B

The rate of red cell destruction in this patient, Case 2, with mild congenital hemolytic anemia (hereditary spherocytosis) was less rapid than in Case 1. There was also a less pronounced but still predominant uptake of Cr^{51} by the spleen.

RESULTS

General observations on red cell survival and calculated daily hemoglobin production

The half-survival of Cr^{51}-labelled autogenous red cells given 3 normal subjects was 33 ± 2 days (mean: 32.8). The red cell survival curves were slightly concave when the uncorrected data were charted on rectilinear graph paper. When these data were corrected for an *in vivo* "elution" of Cr^{51} from Cr^{51}-labelled red cells of 1 per cent daily as suggested by Ebaugh, Emerson, and Ross (12), the normal red cell survival data formed a straight line on rectilinear graph paper, indicating red cell destruction by senescence resulting in a mean cell life span of 112 days. On the other hand in all but 1 of the 11 patients with hemolytic anemias the blood Cr^{51} data uncorrected for elution formed a straight line when plotted against time on semilogarithmic graph paper. Red cell destruction in these subjects was thus "exponential" or random in nature indicating that senescence did not detectably participate in the process, presumably since the rapidity of the random process (or processes) minimized cell death by aging. The rates of red cell destruction were thus calculated directly from the slopes of the red cell survival curves and are given in Table I. The correction

factor for the "elution" of Cr^{51} from normal red cells was again employed, although recognizing that this rate may actually differ for diseased red cells. In one anemic patient, Case 9, despite stable peripheral blood levels during the observation period, the Cr^{51}-labelled red cell survival data corrected for elution produced a straight line on neither rectilinear nor semilogarithmic graph paper. This survival curve presumably reflected a mixture of accelerated senescence and of random destruction. The rate of red cell destruction in Case 9 was estimated by a simple method for determining the mean red cell life span based on the concept of total red cell days (13).

The rates of hemoglobin production shown in Table I were determined in those 10 anemic subjects manifesting random red cell destruction as the product of the calculated rate of red cell destruction and the mean total circulating hemoglobin level during the period of observation. In the normal subjects and in one anemic subject, Case 9, with a mixed hemolytic process hemoglobin production was calculated on the basis of the estimated mean red cell lifespan.

Fate of Cr^{51} derived from labelled red cells, hemoglobin, and chromic chloride

Observations on one of the normal subjects who received Cr^{51}-labelled autogenous red cells are pre-

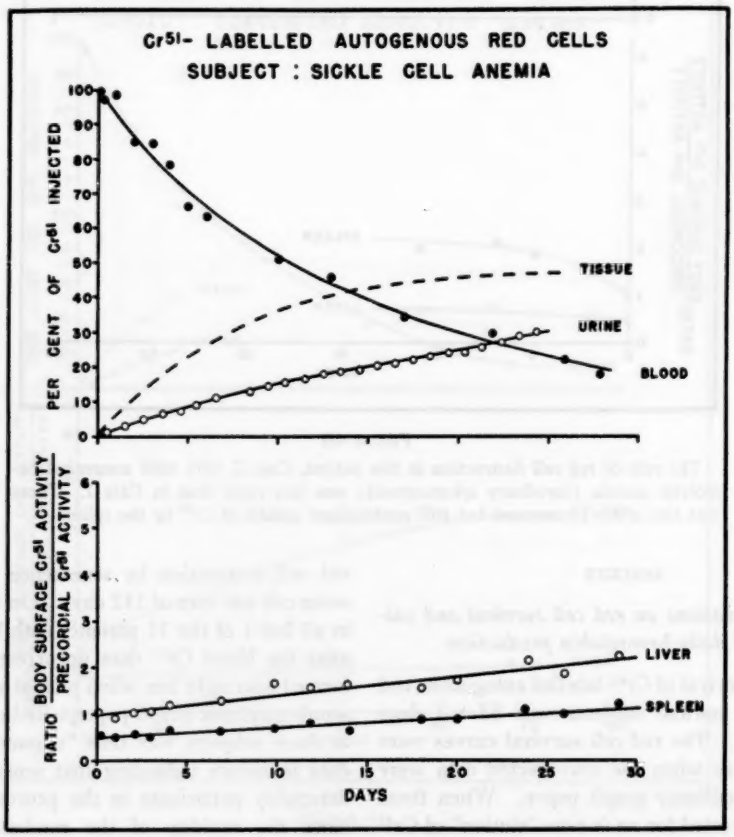

FIGURE 5A

The Cr^{51}-labelled red cells of a patient, Case 3, with moderately severe sickle cell anemia were removed from the circulation in an "exponential" fashion and showed a half-survival time of 11.2 days. There was a moderate increase in radioactivity over the liver but not over the splenic area.

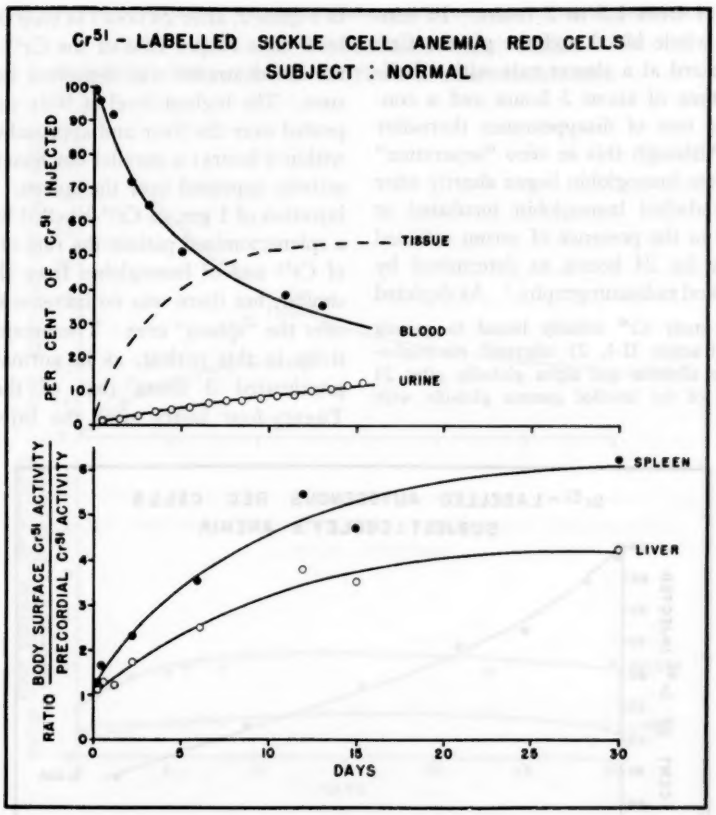

FIGURE 5B

The simultaneously observed survival of red cells from Case 3 in a normal compatible recipient was appreciably shorter with a half-survival time of 6.0 days. This was associated with high levels of radioactivity over both spleen and liver. Note also the smaller excretion of Cr^{51} in the urine of this subject.

sented in Figure 1. In the upper portion slow accumulation of Cr^{51} in the tissues is evident, amounting to about 60 per cent of the Cr^{51} removed from the circulation at the red cell half-survival time. A high, transient Cr^{51} excretion in the urine followed for 1 to 2 days the injection of unwashed Cr^{51}-labelled red cells in subjects not presented here, whether or not a reducing substance such as ascorbic acid had been added to the sample of whole blood prior to injection; however, no such early hyperexcretion of Cr^{51} was evident in the normal subjects who received washed Cr^{51}-labelled red cells. The radioactivity over the livers as well as over the spleens of normal subjects 20 minutes after the injection of Cr^{51}-labelled red cells ranged from 60 to 80 per cent of that over the precordium; and even after 30 days this ratio increased little. The lower portion of Figure 1 depicts the slow minimal increase in relative radioactivity occurring almost equally over liver and spleen thereafter.

Each of three other normal subjects (not listed in Table I) was given 1 gm. of Cr^{51}-labelled autogenous hemoglobin injected intravenously over a period of 2 or 3 minutes. This produced no hemoglobinuria or hemosiderinuria and led to zero-time plasma hemoglobin levels which ranged from 30 to 40 mg. per cent. Hemoglobin, as determined colorimetrically, was cleared from the plasma in an exponential fashion with a half-disap-

pearance time of from 1.5 to 2 hours. In contrast, both the whole blood and the plasma Cr^{51} activity disappeared at a slower rate with a half-disappearance time of about 3 hours and a continually slowing rate of disappearance thereafter (Figure 2). Although this *in vivo* "separation" of chromium from hemoglobin began shortly after injection, Cr^{51}-labelled hemoglobin incubated at 37° C. *in vitro* in the presence of serum retained its radioactivity for 24 hours, as determined by electrophoresis and radioautography.[7] As depicted in Figure 2, after 24 hours *in vivo* 60 per cent, and by 8 days 75 per cent of the Cr^{51} removed from the blood stream was deposited in the body tissues. The highest level of body radioactivity appeared over the liver and approached a maximum within 6 hours; a parallel but lesser rise in radioactivity appeared over the spleen. Following the injection of 1 gm. of Cr^{51}-labelled hemoglobin into a splenectomized patient the rate of disappearance of Cr^{51} and of hemoglobin from the plasma was similar, but there was no increase in radioactivity over the "spleen" area. The maximal hepatic activity in this patient, as in normal subjects, approximated 3 times that of the precordium. Twenty-four hours after the injection of Cr^{51}-

[7] In a similar study Cr^{51} initially bound to gamma globulin (Cohn fraction II-1, 2) migrated electrophoretically with the albumin and alpha globulin after 24 hours' incubation of the labelled gamma globulin with normal plasma.

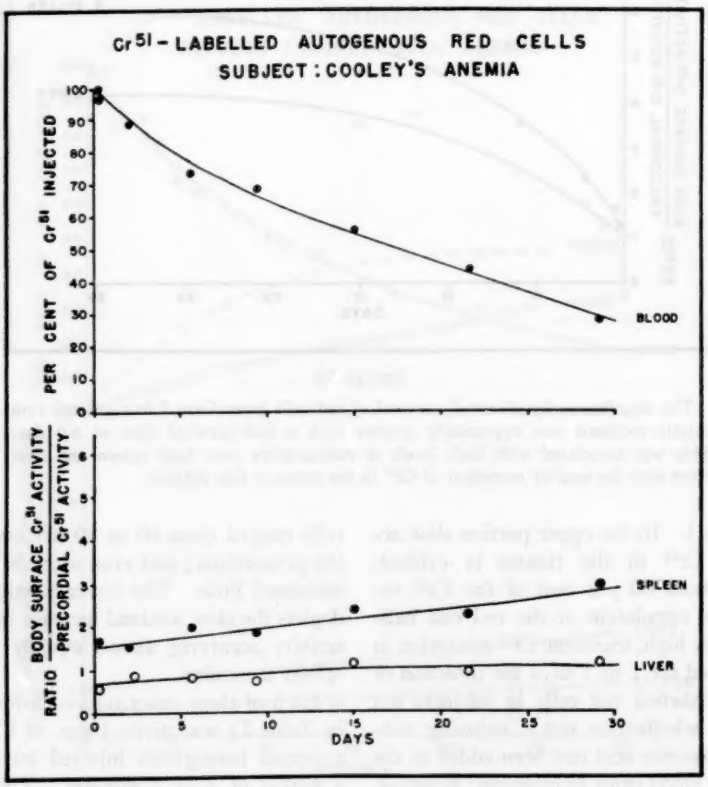

FIGURE 6

The red cell half-survival time in this patient, Case 4, with Cooley's anemia (thalassemia major) was 16.5 days. The high initial relative radioactivity over the patient's large spleen indicates a large vascular organ. The subsequent progressive increase of radioactivity over the patient's spleen mirrored the Cr^{51}-labelled red cell survival curve and indicates progressive red cell sequestration by the spleen.

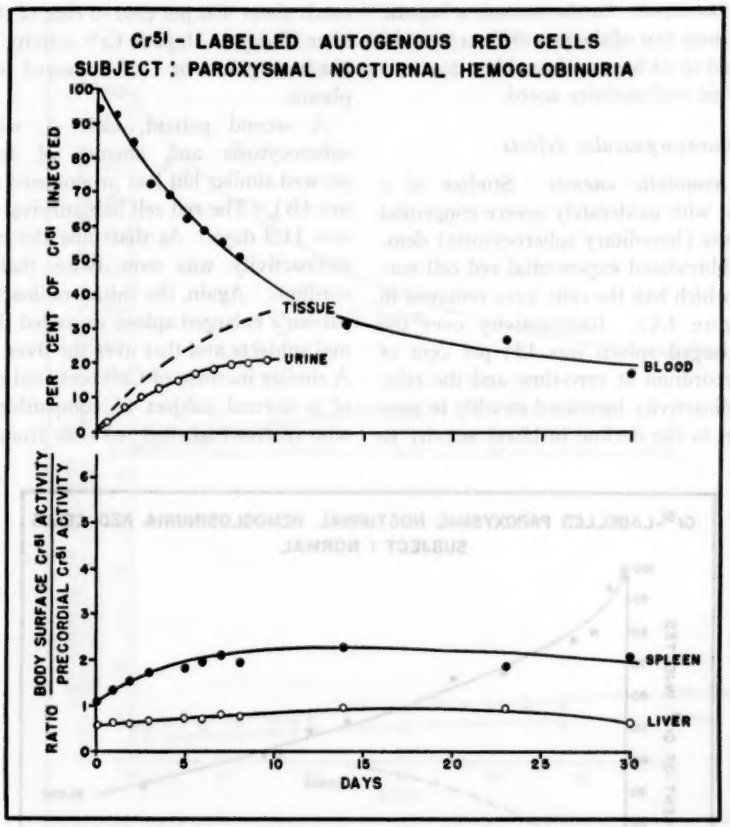

FIGURE 7A

The early rapid phase of red cell destruction depicted in this patient, Case 5, with paroxysmal nocturnal hemoglobinuria and splenomegaly presumably represented destruction of the patient's own red cells with a half-survival estimated at about 7.2 days. The late slow phase was presumed to represent disappearance of the normal previously transfused red cells which were estimated to constitute 30 per cent of those in circulation at the time of labelling with chromium. An increased accumulation of Cr^{51} by the patient's enlarged spleen was observed. This uptake was about half that of a patient with hereditary spherocytic anemia, Case 1, whose red cell survival was similar (Figure 4A).

labelled autogenous hemoglobin solution into a dog, the concentrations of radioactive chromium in the liver and spleen of the sacrificed animal were similar and greatly exceeded those of the lungs, lymph nodes and bone marrow. An intermediate concentration of radioactivity was found in the kidney. The major amount of chromium was thus present in the heaviest organ, the liver.

The fate of a reduction product of sodium chromate, trivalent cationic chromium, was investigated in two normal subjects. In the first subject, $Cr^{51}Cl_3$ was injected in saline intravenously; and in a second subject after preliminary incubation with the recipient's serum at 37° C. for one hour. In both, most of the Cr^{51} was promptly removed from the blood stream and in large part excreted in the urine. The half-disappearance time of Cr^{51} from the blood stream was about 15 minutes in each instance. In the first subject, as shown in Figure 3, a slight progressive hepatic

uptake of Cr^{51} occurred. In the second, a hepatic uptake of two times that of the precordium was observed at the end of 24 hours. In neither case was significant splenic radioactivity noted.

Patients with intracorpuscular defects

Congenital hemolytic anemia. Studies of a patient, Case 1, with moderately severe congenital hemolytic anemia (hereditary spherocytosis) demonstrated an abbreviated exponential red cell survival curve in which half the cells were removed in 8.0 days (Figure 4A). Radioactivity over the moderately enlarged spleen was 124 per cent of that of the precordium at zero-time and the relative splenic radioactivity increased steadily in general proportion to the decline in blood activity to reach about 400 per cent of that of the precordium after 25 days. Hepatic Cr^{51} activity remained low. No hemoglobin or Cr^{51} appeared in the patient's plasma.

A second patient, Case 2, with hereditary spherocytosis and anemia of mild intensity, showed similar but less pronounced findings (Figure 4B). The red cell half-survival in this patient was 11.9 days. At that time the relative splenic radioactivity was over twice that of the precordium. Again, the initial radioactivity over the patient's enlarged spleen exceeded that of the normal subjects and that over the liver remained low. A similar increase of Cr^{51} occurred over the spleen of a normal subject of compatible blood group who received labelled red cells from Case 2. The

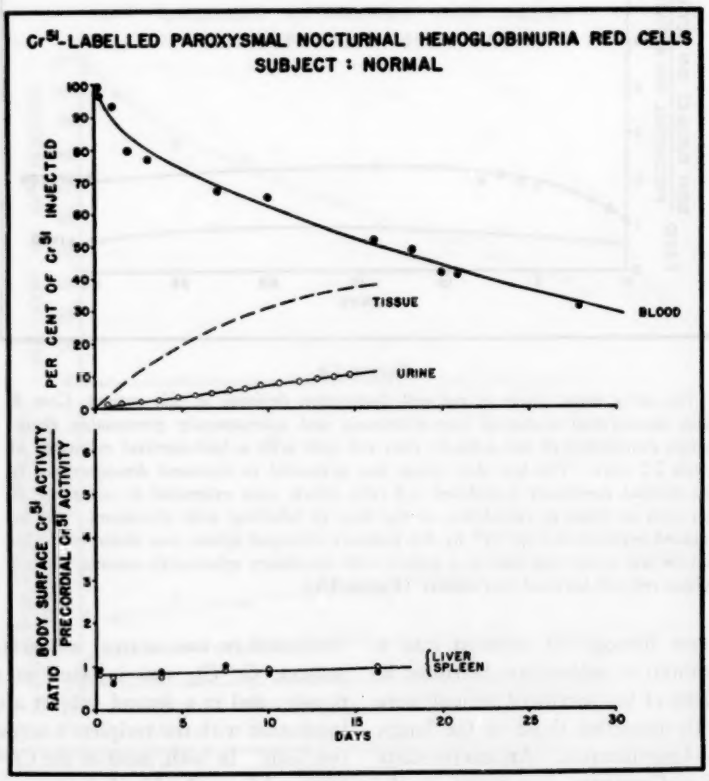

FIGURE 7B

Injection of the red cells of the same patient, Case 5, with paroxysmal nocturnal hemoglobinuria into a normal compatible recipient resulted in a longer red cell half-survival time of these cells, 16.0 days. As indicated above no definite Cr^{51} uptake was evident over either liver or spleen.

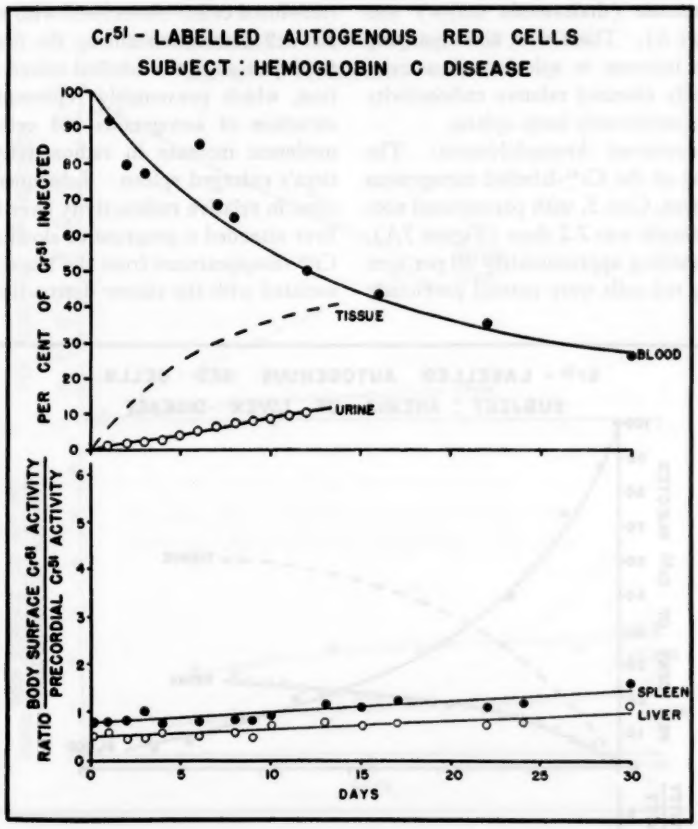

FIGURE 8

The moderate increase in autogenous red cell destruction in a patient, Case 6, with homozygous hemoglobin C disease was associated with only a slight rise in radioactivity over spleen and liver. The Cr^{51} accumulation in the spleen probably falls within normal limits.

patient's red cells were destroyed more rapidly in the normal recipient than in the patient himself.

Sickle cell anemia. The fate of the red cells of a patient, Case 3, with sickle cell anemia is depicted in Figure 5A. Half of these red cells were removed from the blood stream in 11.2 days at which time about 70 per cent of the derived Cr^{51} (35 per cent of that injected) was retained in the body. A moderate gradual increase in hepatic Cr^{51} uptake accompanied the disappearance of Cr^{51} from the blood, whereas "splenic" radioactivity was initially abnormally low and increased little thereafter. A second patient with sickle cell anemia, not reported here, showed almost identical findings. Simultaneously with the study depicted in Figure 5A, a portion of the labelled red cells from Case 3 were injected into a normal compatible recipient. The rate of destruction of the sickle cell anemia red cells was distinctly greater than in the patient herself. Their half-survival time was 6.0 days, and at that time a larger fraction, over 80 per cent, of the derived Cr^{51} was retained in the subject's tissues. In this recipient a pronounced and progressive splenic uptake of Cr^{51} accompanied a moderately heavy increase in hepatic radioactivity. In neither subject did Cr^{51} appear in the plasma.

Cooley's anemia. The half-survival time of the autogenous labelled red cells of a patient, Case 4,

with Cooley's anemia (thalassemia major) was 16.5 days (Figure 6). There was, accompanying this, a moderate increase in splenic radioactivity beyond the initially elevated relative radioactivity over the patient's moderately large spleen.

Paroxysmal nocturnal hemoglobinuria. The half-survival time of the Cr^{51}-labelled autogenous red cells of a patient, Case 5, with paroxysmal nocturnal hemoglobinuria was 7.2 days (Figure 7A). At the time of labelling approximately 30 per cent of the circulating red cells were normal previously transfused cells. Associated with a period of rapid red cell destruction during the first 15 days after the injection of the labelled mixed red cell population, which presumably represented largely destruction of autogenous red cells, there was a moderate increase in radioactivity over the patient's enlarged spleen. Subsequently a slight decline in relative radioactivity over both spleen and liver attended a progressive slowing of the rate of Cr^{51} disappearance from the blood presumably associated with the slower destruction of the normal

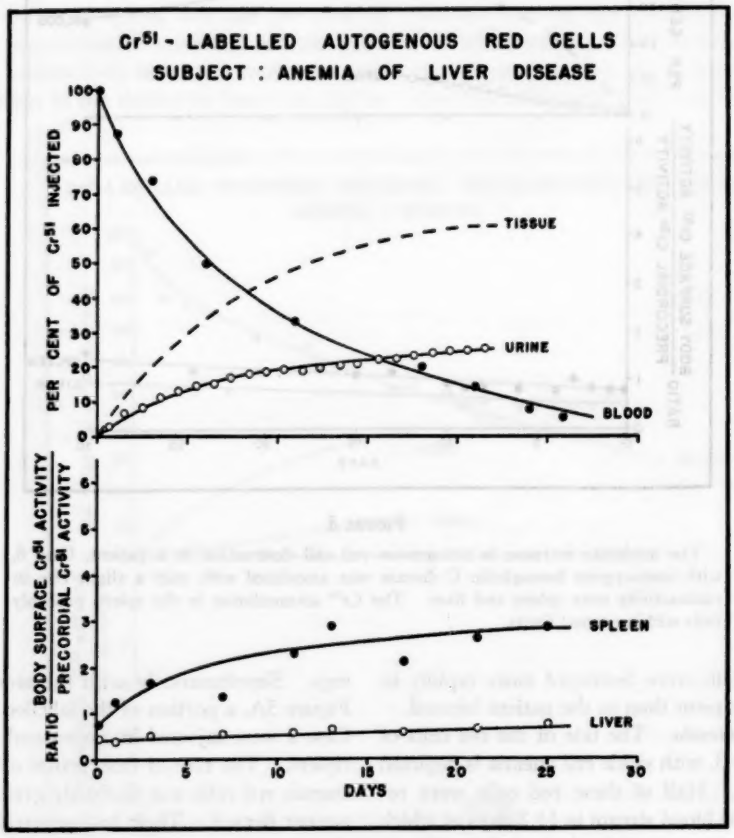

FIGURE 9

In this patient, Case 7, with severe Laennec's cirrhosis and macrocytic anemia associated with chronic alcoholism the increased rate of disappearance of Cr^{51} from the blood indicated a moderately severe hemolytic process characteristic of this disease. This was associated with a distinct and progressive accumulation of Cr^{51} over the patient's non-palpable spleen. Note also the initial and sustained abnormally low relative radioactivity over the patient's enlarged liver. This was observed in 3 other patients with cirrhosis, and presumably reflects a reduction in hepatic vascularity.

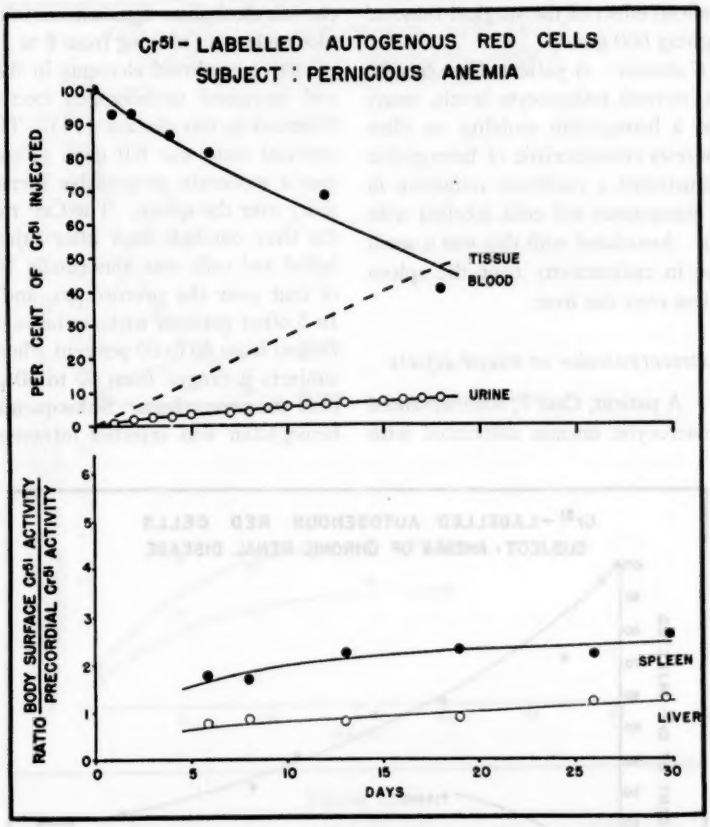

FIGURE 10

The survival time of the red cells of a patient, Case 8, with untreated pernicious anemia was moderately reduced. The moderate increase in radioactivity over the patient's non-palpable spleen indicates that the red cells were largely sequestered in that organ.

transfused red cells. One hour after the injection of the labelled red cells 0.6 per cent of the whole blood radioactivity resided in the patient's plasma indicating prompt release of labelled hemoglobin from destroyed red cells. Plasma radioactivity increased to 4.7 per cent of that in the whole blood 1 day later, remained at about 5 per cent for 4 more days and then gradually diminished. The ratio of Cr^{51} to hemoglobin in the plasma was several times greater than that in the red cells. No plasma Cr^{51} activity remained at 14 days. In contrast to the deposition of Cr^{51} from labelled red cells largely sequestered in the spleen, the injection into this patient of Cr^{51}-labelled hemoglobin (not shown in Figure 7A) led to an increase in relative radioactivity over both the liver and spleen, with the hepatic uptake predominating slightly. The half-survival time of the Cr^{51} derived from labelled hemoglobin was about three and one-half hours.

Labelled red cells from Case 5 were destroyed less rapidly when transfused into a normal compatible subject (Figure 7B). In this instance the red cell half-survival time was 16.0 rather than 7.2 days, and there was no apparent sequestration by either spleen or liver. Plasma radioactivity was not measured. The possibility, implied by these results, of an exaggerated red cell sequestering action by the patient's clinically somewhat enlarged spleen superimposed upon the underlying intravascular hemolytic process was borne out by the

subsequent beneficial effect of the surgical removal of a spleen weighing 660 gm.

Hemoglobin C disease. A patient, Case 6, with a slight anemia, normal reticulocyte levels, many target cells, and a hemoglobin mobility on filter paper electrophoresis characteristic of hemoglobin C disease, demonstrated a moderate reduction in the survival of autogenous red cells labelled with Cr^{51} (Figure 8). Associated with this was a small gradual increase in radioactivity over the spleen and somewhat less over the liver.

Patients with extracorpuscular or mixed defects

Liver disease. A patient, Case 7, with advanced cirrhosis and macrocytic anemia associated with chronic alcoholism demonstrated the elevated reticulocyte levels (ranging from 8 to 24 per cent), hyperactive erythroid elements in the bone marrow, and increased urobilinogen excretion frequently observed in this disease (14). The red cell half-survival time was 6.0 days (Figure 9). There was a moderate progressive increase of radioactivity over the spleen. The Cr^{51} radioactivity over the liver one-half hour after injection of the labelled red cells was abnormally low, 43 per cent of that over the precordium, and remained low. In 3 other patients with cirrhosis this initial value ranged from 40 to 60 per cent whereas in 6 normal subjects it ranged from 60 to 80 per cent of that over the precordium. Subsequently, Cr^{51}-labelled hemoglobin was injected intravenously into this

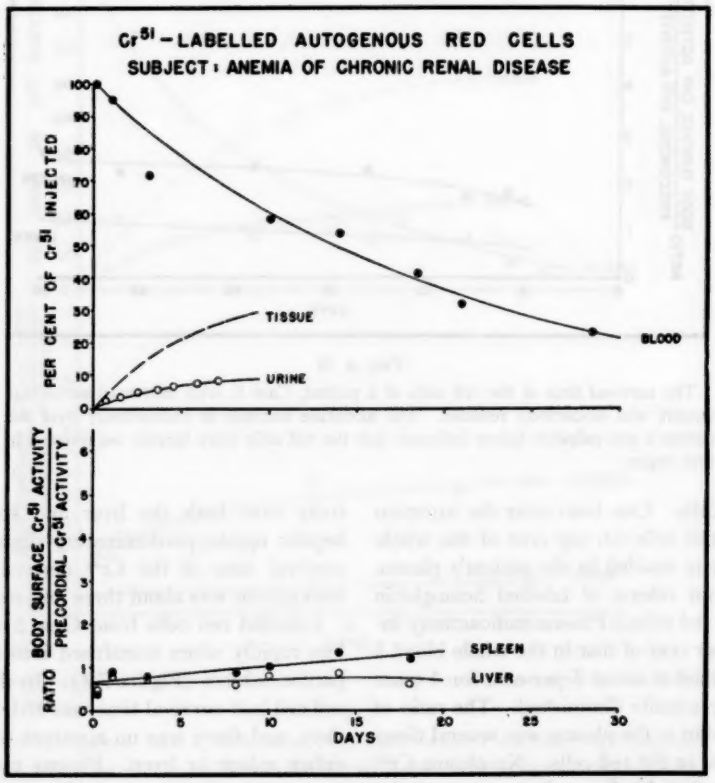

FIGURE 11

This patient, Case 9, had a fairly severe, stable anemia associated with uremia resulting from chronic pyelonephritis. Despite a Cr^{51}-labelled red cell half-survival of only 14.4 days, no abnormal accumulation of Cr^{51} occurred in either liver or spleen.

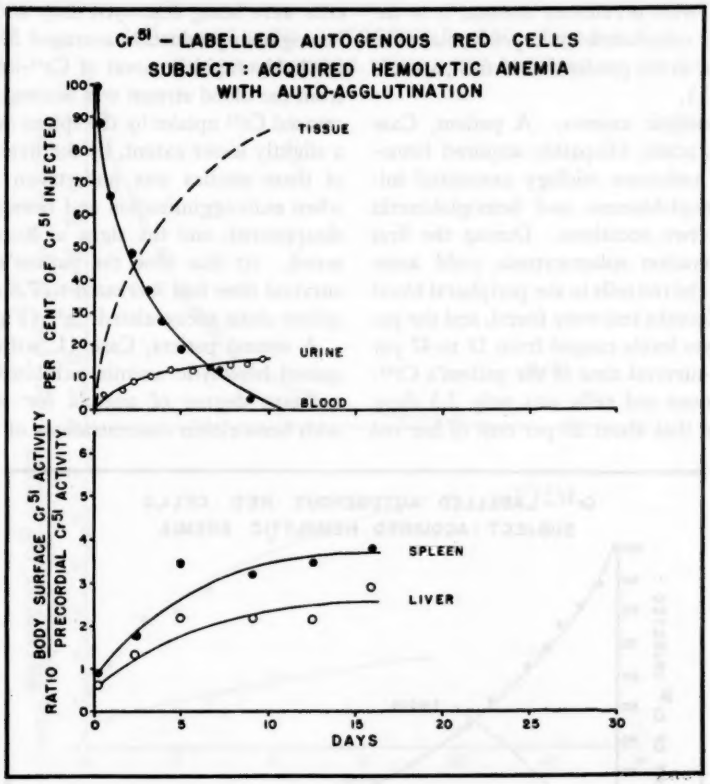

FIGURE 12A

The extremely rapid disappearance of Cr^{51}-labelled red cells in a patient, Case 10, with a fulminating hemolytic process of unknown etiology was associated with an increased tissue retention of Cr^{51} and with high levels of radioactivity over both spleen and liver. Autoagglutination of the red cells in the peripheral blood and hemoglobinemia were present.

subject in divided daily doses calculated to approximate the amount of hemoglobin which had been released from the Cr^{51}-labelled red cells each day. The Cr^{51} injected in this manner accumulated largely in the patient's liver rather than in the spleen.

Pernicious anemia. The survival time of red cells from patients with untreated pernicious anemia when transfused into normal subjects has been observed by the Ashby technique to be diminished (15, 16). A reduced survival of red cells labelled with Cr^{51} was also observed here in a patient, Case 8, with moderately severe untreated pernicious anemia (Figure 10). A moderate accumulation of Cr^{51} in the patient's non-palpable spleen was also demonstrated whereas there was no definite uptake of Cr^{51} by the liver.

Anemia of chronic renal disease. A patient, Case 9, with chronic "stationary" uremia had a sustained, moderately severe, normocytic, normochromic anemia. The reticulocyte levels ranged from 1 to 4 per cent; there were about 5 per cent of "burr cells" in the peripheral blood, and the bone marrow aspirate was normal in appearance and cellular composition. The patient was found to have a reduced survival time of Cr^{51}-labelled autogenous red cells (Figure 11), a frequent finding in uremia (17–19). No increased Cr^{51} uptake by either liver or spleen attended this process. Despite the shortened red cell survival, as in the

patient, Case 8, with pernicious anemia, it is interesting that by calculation underproduction was the greater factor in the production of this patient's anemia (Table I).

Acquired hemolytic anemia. A patient, Case 10, with severe, acute, idiopathic acquired hemolytic anemia of unknown etiology associated initially with hemoglobinemia and hemoglobinuria was studied on two occasions. During the first period of observation spherocytosis, mild autoagglutination of the red cells in the peripheral blood and a positive Coombs test were found, and the patient's reticulocyte levels ranged from 17 to 47 per cent. The half-survival time of the patient's Cr^{51}-labelled autogenous red cells was only 2.3 days. It was estimated that about 29 per cent of her red cells were being destroyed daily and that the daily hemoglobin production averaged 55 gm. This remarkably rapid removal of Cr^{51}-labelled red cells from the blood stream was accompanied by an increased Cr^{51} uptake by the spleen and also, to only a slightly lesser extent, by the liver. A repetition of these studies was undertaken 3 weeks later, when auto-agglutination and hemoglobinemia had disappeared, and the signs of hemolysis had lessened. At this time the patient's red cell half-survival time had increased to 7.8 days, while the spleen alone accumulated Cr^{51} (Figure 12B).

A second patient, Case 11, with idiopathic acquired hemolytic anemia exhibited a remarkably constant degree of anemia for several months, with hemoglobin concentrations of from 6.0 to 7.0

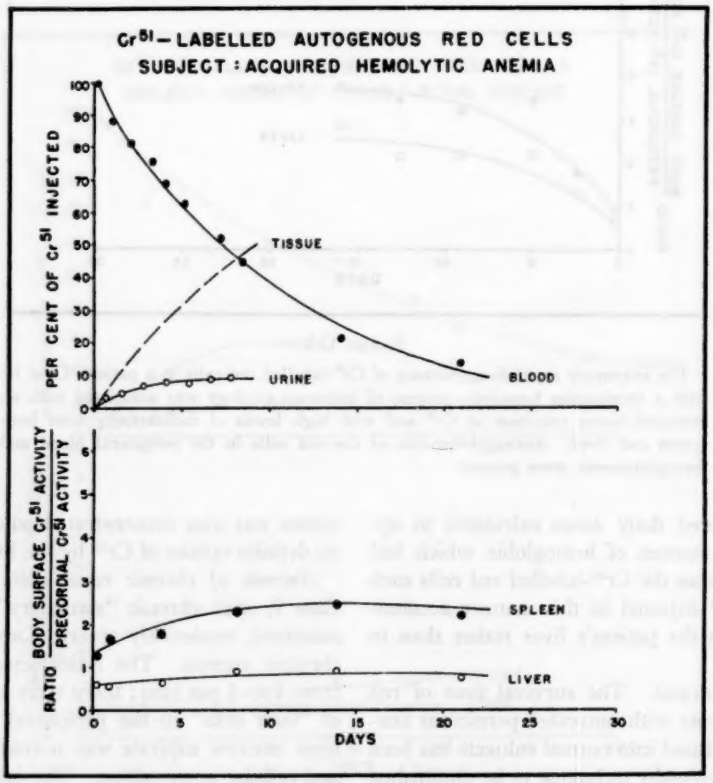

FIGURE 12B

Repetition 3 weeks later of the injection of Cr^{51}-labelled autogenous red cells revealed an improved red cell survival at a time when the anemia had diminished. At this time autoagglutination and hemoglobinemia had disappeared, but there was a persistence of red cell sensitization, and less striking evidence of red cell sequestration which was now confined to the spleen.

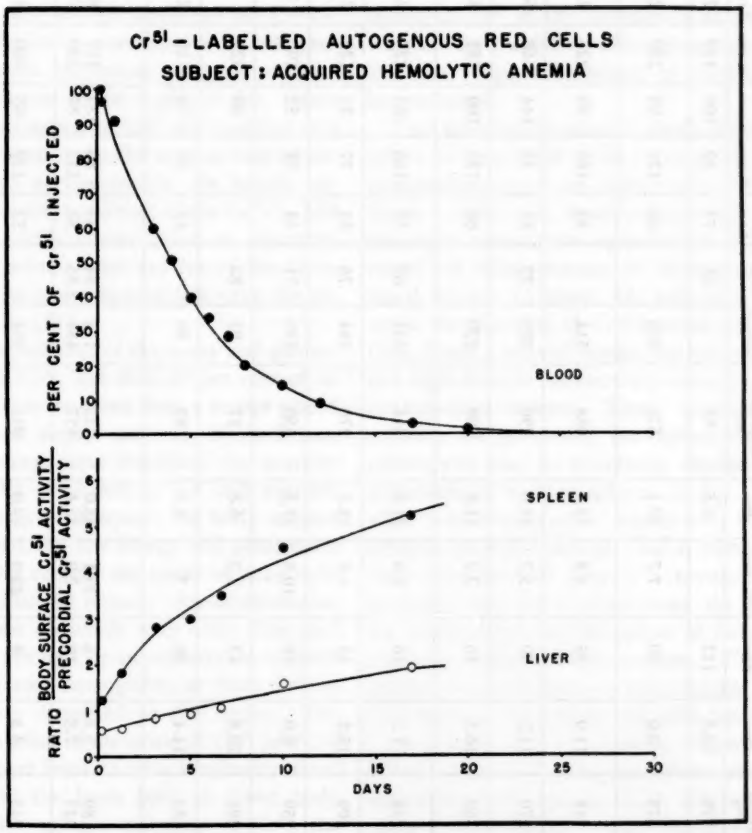

FIGURE 13

In this patient, Case 11, with moderately severe, stable idiopathic acquired hemolytic anemia associated with evidence of red cell sensitization approximately 20 per cent of the patient's Cr^{51}-labelled red cells were removed from the circulation daily. A heavy accumulation of Cr^{51} in the spleen mirrored the rapid disappearance of Cr^{51} from the peripheral blood.

gm. per cent and reticulocyte levels ranging from 19 to 34 per cent. The direct and indirect Coombs tests were persistently positive and slight hemoglobinemia (10 to 15 mg. per cent) continued to be present. An estimated 20 per cent of the patient's Cr^{51}-labelled red cells were destroyed daily and high levels of radioactivity accumulated over the slightly enlarged spleen (Figure 13).

DISCUSSION

Cr^{51} in the determination of the sites of red cell sequestration

The observations made on the normal subjects indicate that the excretion and body distribution of Cr^{51} derived from red cells labelled with $Na_2Cr^{51}O_4$ differs characteristically from that of Cr^{51} derived from Cr^{51}-labelled hemoglobin and from saline solutions of $Cr^{51}Cl_3$. The tissue concentrations in the dog's liver and spleen of Cr^{51} derived from Cr^{51}-labelled hemoglobin were similar. Thus, the comparatively large liver contained a larger total amount of Cr^{51}. This presumably explains the high radioactivity values found over the livers of normal human subjects who were injected with labelled hemoglobin. A comparable distribution of radioactivity between liver and spleen in rats was observed by Finch and his associates (20) with Fe^{59}-labelled hemoglobin.

TABLE I

The red cell survival, daily hemoglobin production, and Cr^{51} distribution between liver and spleen of 11 patients with various types of anemia

Case	Diagnosis	Sex	Age	Weight *Kg.*	Estimated organ size, cm. below costal margin		Hemoglobin *gm. %*	Blood volume *cc.*	Total circulating hemoglobin *gm.*	Red cell half-survival time *days*	Estimated mean red cell lifespan* *days*	Rate of red cell destruction* *%/day*	Estimated hemoglobin production*			Tissue deposition of Cr^{51}† %	Relative Cr^{51} activity,‡ %				Index of sequestration§	
																	R_0		R_{10}		$R_{10}-R_0$	
					Liver	Spleen							*gm./day*	*mgm./Kg. body wgt./day*	*% normal*		Liver	Spleen	Liver	Spleen	Liver	Spleen
	Normals (3)			68.5	0	0	14.8	4,920	728	32.8	112		6.5	95		61	73	67	100	110	27	43
1	Congenital hemolytic anemia	M	66	50.5	1	13	9.4	4,020	378	8.0	20	7.7	29.1	576	606		66	124	90	250	24	126
2	Congenital hemolytic anemia	M	18	73.6	0	3	13.9	5,381	748	11.9	35	4.8	35.9	488	514		53	105	80	225	27	120
3	Sickle cell anemia	F	17	48.2	0	0	8.8	3,070	270	11.2	30	5.2	14.0	290	305	72	84	54	144	68	60	14
4	Cooley's anemia	F	33	56.4	3	15	8.2	4,498	369	16.5	46	3.2	11.8	209	220		68	155	100	252	32	97
5	Paroxysmal nocturnal hemoglobinuria‖	M	49	69.5	0	3	4.6	5,530	254	7.2	16	8.6	21.8	314	331	62	55	106	83	225	28	119
6	Hemoglobin C disease	M	66	67.7	0	0	12.5	4,074	509	15.2	35	3.6	18.3	270	284	76	52	77	75	122	23	55
7	Anemia of liver disease	F	55	50.0	4	0	7.1	3,650	259	6.0	16	10.6	27.5	550	579	71	43	78	52	190	9	112
8	Pernicious anemia	F	64	77.7	0	0	5.5	5,343	294	20.8	43	2.3	6.8	87	92	83			90	225		
9	Anemia of uremia	F	55	52.7	0	0	6.0	2,550	153	14.4	36	¶	4.3	82	86		57	58	78	110	21	52
10	Acquired hemolytic anemia																					
	(a) Acute	F	31	75.0	3	1	4.4	4,300	189	2.3	6	29.1	55.0	733	772	84	53	80	133	187	80	107
	(b) Subacute	F	31	73.1	0	0	10.3	4,110	423	7.8	19	7.9	33.4	457	481	85	58	112	84	220	26	108
11	Acquired hemolytic anemia	F	50	51.9	0	1	6.7	3,662	245	3.3	8	20.0	49.0	944	994		52	120	95	300	43	180

* In the calculation of the lifespan and of the rate of random destruction of red cells and of the rate of hemoglobin production a correction factor of 1 per cent per day was employed to compensate for "Cr^{51} elution" from the red cells *in vivo* (12).

† The percentage of the injected Cr^{51} removed from the blood stream but not excreted in the urine at the red cell half-survival time.

‡ The Cr^{51} activity over the described organ is expressed here as a percentage of that over the precordium; the values recorded were determined at zero-time (R_0) and at the red cell half-survival time (R_{10}).

§ As described in the text this is a numerical designation representing the relative radioactivity at the red cell half-survival time (R_{10}) minus that present at zero-time (R_0).

‖ Approximately 30 per cent of the circulating red cells in this patient at the time of these observations were normal transfused cells; a correction was made in the above calculations providing an estimate of actual endogenous hemoglobin production.

¶ The red cell survival curve in this subject was not that of purely random processes; the rate of hemoglobin production was thus estimated, as in the normal subjects, on the basis of the mean red cell lifespan.

The observed very abrupt clearance of $Cr^{51}Cl_3$ from the circulation is consistent with the propensity of cationic chromium to form colloidal molecular aggregates above a pH of 6.0. Here again, the small fraction of Cr^{51} not excreted was predominantly taken up by the normal liver as determined by body surface counts; the hepatic uptake of Cr^{51} was more striking when $Cr^{51}Cl_3$ had been incubated with serum prior to injection. These results conform with studies of the chromium distribution in rat tissues following the injection of $Cr^{51}Cl_3$ (21).

Because of the softness of the x-ray and gamma ray emissions of Cr^{51}, less than 20 per cent of its radiations penetrate the trunk from a source placed beneath a normal supine subject. Nevertheless, with use of the apparatus described, the quantity of Cr^{51} generally employed in red cell survival studies (12) is quite adequate for body surface counting. Indeed, the low energy and penetration of these emissions reduces the factor of stray radiation from neighboring organs. An accumulation of local radiation obviously may arise from red cells in transit through or sequestered in a given organ, from released hemoglobin or from Cr^{51} in some other form deposited in the organ. The gradual accumulation in the urine of Cr^{51} presumably results in part from its slow elimination from the tissues. On the basis both of direct body counts and of the urinary excretion of Cr^{51}, the rate of loss from the tissue was calculated in 4 subjects who had abruptly sequestered labelled sensitized red cell suspensions (9) to average about 4 per cent per day; the half-life for tissue elimination of Cr^{51} ranged from 14 to 21 days. Excretion of Cr^{51} was entirely renal. In no instance was there measurable Cr^{51} in the stool. Stool Cr^{51} measurements thus permit the ready detection of a potential error in red cell survival studies: occult intestinal bleeding.

The findings in normal subjects when compared with those in the anemic patients presented above justify certain limited conclusions from body surface counting following injection of red cells labelled with radioactive sodium chromate: (a) A progressive, high splenic uptake of Cr^{51} represents the splenic sequestration of intact red cells. There appears to be no other way by which Cr^{51} from labelled red cells can be accumulated preponderantly by the spleen. (b) A high hepatic uptake of Cr^{51} in the presence of an intact spleen suggests the possibility, although not the certainty, that intravascular hemolysis of labelled red cells has occurred.

That the sequestration of whole red cells by the spleen is indeed detectable by an attendant rise in radioactivity over the spleen was illustrated by Cases 1 and 2. In these patients with congenital hemolytic anemia the splenic Cr^{51} uptakes mirrored the disappearance of chromium from the blood stream (Figures 4A and 4B). Furthermore, the injection of Cr^{51}-labelled red cells from Case 2 into a normal compatible recipient also led to a high relative radioactivity over the recipient's normal-sized spleen. These observations corroborate the previously established evidence that spherocytes may be selectively retained by either pathologic or normal spleens (1–5). The somewhat longer survival of autogenous Cr^{51}-labelled spherocytes in the patient, Case 2, with mild hereditary spherocytosis than in a normal compatible recipient may have arisen from the competition for sequestration in the spleen of Case 2 by autogenous unlabelled spherocytes. The moderate hepatic and comparatively low "splenic" uptake of Cr^{51}-labelled autogenous red cells from a patient, Case 3, with sickle cell anemia (Figure 5A) were interpreted as reflecting the splenic atrophy characteristic of this disease (6, 7, 22), especially because prominent progressive increases in splenic as well as hepatic radioactivity were observed when an aliquot of the sickle cell anemia patient's labelled red cells was transfused into a compatible normal subject (Figure 5B). That the sicklemia red cells survived only half as long in the normal subject as in the patient herself presumably indicates in part the beneficial effect on red cell survival of the "autosplenectomy" characteristic of adult patients with sickle cell anemia. This is in contrast to the short survival time, even of normal red cells, in some children with sicklemia and large spleens observed by Lichtman, Shapiro, Ginsberg, and Watson (23). The considerable hepatic Cr^{51} uptake noted here also contrasts with the preponderant uptake of Cr^{51} by the spleen alone in congenital hemolytic anemia. These observations, again, are consistent with previous studies (1, 5–7) revealing the hematologic basis for the erythrostasis, thromboses and infarcts

which early cause severe damage to the spleen and which gradually injure the liver.

In addition to establishing the extent to which Cr^{51} is accumulated by the liver or spleen, it is possible to discriminate by means of their respective rates of increase between high relative radioactivity due on the one hand to sequestration of labelled red cells and that on the other due to simple organ enlargement with concomitant increase in vascularity. In the former situation local radioactivity necessarily builds up in an organ in approximate proportion to its rate of disappearance from the blood. In the latter situation the high initial relative activity over the organ (unless followed at once by rapid accumulation) signifies a large vascular bed rather than red cell sequestration. Thus, the presence of a high initial ratio of spleen Cr^{51} to precordial Cr^{51}, *i.e.*, greater than about 90 per cent, merely indicates splenomegaly with an attendant increase in perfusing blood and unless the ratio subsequently increases, as for example in Cases 1, 2, 4, 5, 7, 10 and 11, does not necessarily denote sequestration of red cells. A case illustrating this point is presented in Figure

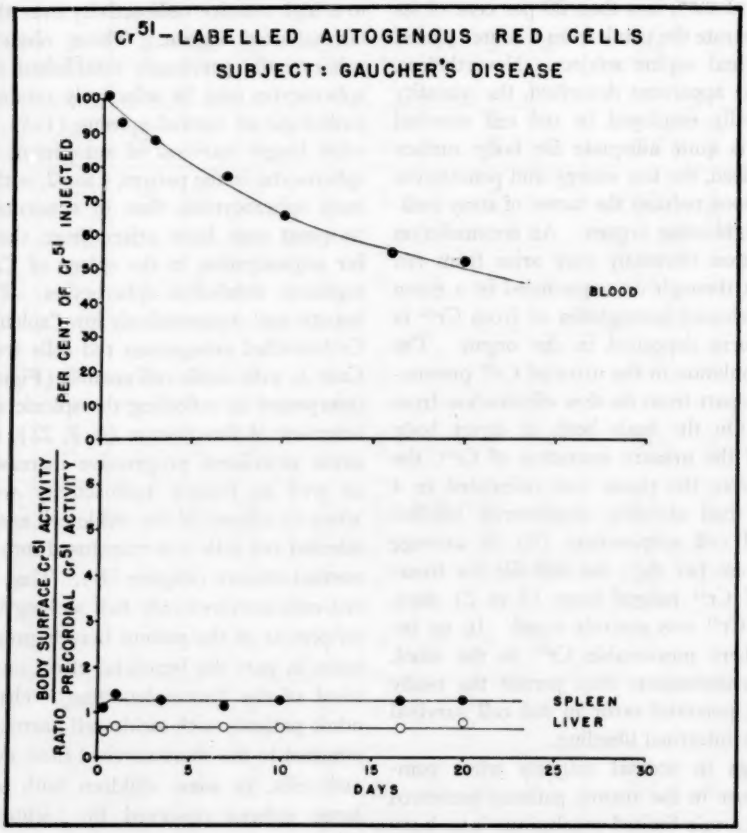

FIGURE 14

This 48-year old Jewish female had splenomegaly and thrombocytopenia on the basis of Gaucher's disease. The patient was not anemic, however, and her red cell survival, as indicated above, was almost normal. Although a high relative radioactivity appeared in the enlarged spleen shortly after the injection of Cr^{51}-labelled autogenous red cells, there was no subsequent accumulation of Cr^{51} by the spleen. This pattern of radioactivity reflects the vascular size of the spleen rather than the filtration of red cells.

14. This patient had splenomegaly on the basis of Gaucher's disease and showed no evidence of hemolysis. Her red cell half-survival time was about 25 days. Within a few hours of the injection of Cr^{51}-labelled autogenous red cells the radioactivity over the spleen was 125 per cent of that over the heart; as blood Cr^{51} activity declined, however, there was no further uptake of radioactivity by the spleen, suggesting that this large spleen possessed a large vascular bed but did not sequester red cells. Accordingly, Cr^{51} may sometimes be employed to determine the nature of masses in the left upper quadrant when this is not otherwise possible. It must be pointed out, however, that splenomegaly may exist with little or no increase in relative radioactivity over the spleen. Thus, whereas the initial Cr^{51} activity over the spleen was elevated in all observed cases of splenomegaly associated with a hemolytic anemia or usually with cirrhosis, this was not so, despite the presence of marked splenomegaly, in one case of chronic lymphatic leukemia; and the Cr^{51} activity was only slightly increased in one case of agnogenic myeloid metaplasia with massive splenomegaly. Presumably in these 2 patients splenomegaly reflected non-vascular cellular hyperplasia. The use of Cr^{51}-labelled normal red cells in the anatomic identification of the spleen is thus reliable only in a positive sense, that is, when a high initial relative radioactivity occurs over the suspected area. Conceivably sufficiently large vascular tumors, such as hemangiomata of the liver, might be similarly identified.

Observations (9) reported elsewhere upon the organ distribution of Cr^{51} and the appearance of hemoglobin in the plasma following injection of Cr^{51}-labelled red cells after exposure to complete or to incomplete antibodies indicate the experimental association of red cell agglutination and deposition in the lung and liver with hemoglobinemia. On the other hand, the association of red cell sensitization, without manifest agglutination, was with splenic sequestration and the absence of hemoglobinemia. On the basis of the plasma hemoglobin levels these processes have classically been distinguished as "intravascular" and "extravascular" hemolysis. The former state has often been taken to indicate the presence of a circulating hemolysin despite the rarity with which hemolysins are actually demonstrable in hemolytic diseases, even in patients (such as Case 10) with fairly striking increases in plasma hemoglobin levels. These experimental observations and those clinical observations reported or referred to here indicate the general tendency of mild or of discrete red cell alterations (as encountered in spherocytes, sensitized cells, and the morphologically peculiar cells encountered in pernicious anemia and in Cooley's anemia) to lead to rather specific sequestration by the spleen presumably due to the refined filtering function of that organ. This is attended by little or no release of hemoglobin into the circulation and may be interpreted as an "extravascular" process. More severe or gross red cell changes, such as agglutination, or discrete cellular changes which may grossly affect blood viscosity, as in sickle cell anemia, lead to a more indiscriminate filtration by the capillaries, especially in the several organs with very large vascular beds: the lungs and liver, as well as the spleen if present and functioning. Such widespread red cell trapping, especially when not in close relation to concentrations of reticulo-endothelial cells, may favor the release of hemoglobin into the plasma and thus simulate the effects of a circulating hemolysin.

It is interesting to note the coexistence of red cell agglutination, a large hepatic uptake of Cr^{51}, and high plasma hemoglobin levels at the time of the first observation on Case 10, a patient at that time with fulminating acquired hemolytic anemia. In Case 11, with an acquired hemolytic anemia of slightly less severity, the red cells were sensitized but not agglutinated, the preponderance of Cr^{51} activity in the spleen was striking, and there was little rise in the plasma hemoglobin level. Of further interest was the observation that Case 5, the patient with paroxysmal nocturnal hemoglobinuria, manifested an increase in hemoglobinuria following splenectomy despite improvement in the red cell hemoglobin concentration. Later still iron deficiency ensued as evidenced by a fall in mean corpuscular hemoglobin concentration and in the serum iron level. These observations upon Case 5 suggest that following removal of the spleen a greater proportion of the patient's cells were destroyed intravascularly rather than in the spleen with consequent increase in the urinary excretion of iron.

Technical hazards in the use of Cr^{51}

In the clinical application of the present method, the Cr^{51}-labelling techniques described by others (12) are generally suitable. The uptake of chromate by red cells is highly pH-dependent, diminishing sharply as the pH exceeds 7.0. Thus, acid anticoagulant solutions, such as ACD solution, are suitable for red cell labelling with sodium chromate.[8] In addition, trivalent chromium arising from reduction of the chromate by dextrose or by added ascorbic acid is bound by citrate, which in turn inhibits binding by the plasma proteins and by the red cell membranes (9). During the initial moments of the labelling process, chromate enters the red cells so rapidly that a disproportionate "loading" of a small fraction of the cells occurs unless precautions are taken to employ a dilute solution of sodium chromate and to secure its immediate and thorough mixture with the blood sample. Chromate is an oxidant relative to hemoglobin, and methemoglobin appears in detectable quantities when the molecular ratio of chromate to hemoglobin exceeds 0.1, *i.e.*, above about 22 micrograms of Cr^{51} or about 70 micrograms of $Na_2Cr^{51}O_4$ per cubic centimeter of packed red cells.[9] The glycolysis of red cells, as measured in the Warburg apparatus, was found to be depressed in arithmetic proportion to the chromate concentration of the suspension. A significant depression of red cell glycolysis was demonstrable following the addition of as little as 10 micrograms of Cr^{51} in the form of sodium chromate per cubic centimeter of packed red cells. Chromated hemoglobin was observed to migrate more rapidly than does normal hemoglobin toward the anode of a filter paper electrophoresis cell maintained at a pH of 8.6 by barbital buffer. Under these conditions, the greater negative charge of the Cr^{51}-labelled hemoglobin induced an increase in the electrophoretic mobility of hemoglobin of over 15 per cent. All of these observations stress the chemical alterations brought about by the Cr^{51}-labelling procedure, and urge that care be taken to label the red cell aliquots evenly and with $Na_2Cr^{51}O_4$ of high specific radioactivity.

Ordinarily there is no apparent necessity for washing the patient's red cells prior to re-injection or for measuring the urinary excretion of Cr^{51}. The chief advantage of washing the red cells prior to injection is to obviate an initial urinary hyperexcretion of Cr^{51} which is not derived from labelled red cells but from the plasma. The injection of a small amount of Cr^{51} unattached to red cells will also impair the accuracy of the initial blood volume determinations if whole blood specimen radioactivity is measured, but it should not interfere with the assessment of splenic sequestering activity.

The extensive accumulation of Cr^{51} observed to occur in the spleens and livers of patients with hemolytic anemias necessitates a re-appraisal of the radiation hazard involved. The radiation dosage contributed by the injection of 100 μC of Cr^{51} into a 70 Kg. subject has been estimated to be about 0.1 rep, with the assumption that the isotope is evenly distributed within the circulation and that no excretion has occurred (24). In a patient with a hemolytic process in which the injected Cr^{51} is accumulated entirely by the liver and spleen, with combined organ weights of 1600 grams, the exposure to these tissues approximates 2.0 rep. If a 100 μC dose of Cr^{51} is accumulated entirely by a 200-gram spleen, the exposure to that organ will approximate 16.1 rep, an excessive level. Although total clearance of Cr^{51} by the spleen is unlikely, it is suggested that the dose of Cr^{51} employed in subjects with presumed hemolytic anemias be reduced to a maximum of 50 μC.

Cr^{51} in predicting the need for splenectomy

After each injection of labelled red cells determination of the body surface Cr^{51} activity by means of three or four appropriately spaced sets of observations over precordium, liver, spleen and

[8] An exception to this was made in studying the red cells of the patient with paroxysmal nocturnal hemoglobinuria. In order to avoid the acid hemolysis characteristic of this patient's red cells, heparin was employed as an anticoagulant during the labelling procedure.

[9] This reaction appears to be a direct chemical oxidation of hemoglobin progressing in proportion to dosage and to time equally well with hemoglobin solutions or with intact red cells. That inactivation of the enzyme normally concerned with the reduction of methemoglobin is not involved was suggested by study of the effect of chromate upon the red cells of a patient with congenital methemoglobinemia, a disease in which this enzyme is believed to be absent or deficient: the rate and extent of conversion of oxyhemoglobin in the patient's red cells to methemoglobin by chromate was the same as in normal red cells.

thorax should suffice to establish the sites of red cell sequestration. For correlation with the rates of disappearance of Cr^{51}-labelled red cells from the peripheral blood it is desirable to perform body surface counts for at least half the red cell survival period. A suggested procedure is to make a set of observations 20 or 30 minutes after the injection of labelled red cells and again one day later. Two or three more observations of body surface radioactivity (for example, after an estimated 25 per cent, 50 per cent, and 90 per cent, respectively, of the labelled cells have been destroyed) should provide the desired information. Employment of a uniform, geometric positioning of patients and of counting apparatus at the time of each observation is, of course, essential to obtaining reproducible data. Nevertheless, a portable counting unit at the bedside should be sufficiently accurate to determine the approximate distribution of radioactivity between heart, liver, and spleen.

The *in vivo* determination of the site of red cell sequestration should prove of greatest practical value in predicting the need for splenectomy in acquired hemolytic anemias, wherein the greatest prognostic uncertainty presently exists. Interpretation of the data obtained by the methods described may be simplified by using a standardized expression such as the difference between the relative radioactivity (expressed as a percentage of that over the precordium) for liver or spleen at the red cell half-survival time (R_{50}) and such values at zero time (R_0). A simple formula may then be invoked as an index of red cell sequestration as follows:

$$\text{Index of Sequestration} = R_{50} - R_0$$

In 3 normal subjects, these values for the liver ranged from 23 to 35 and for the spleen from 38 to 48 with average values of 27 and 43, respectively, as recorded in Table I. A tentative grading of the intensity of splenic sequestration on the basis of the index of sequestration values recorded in Table I for the 11 anemic subjects might be as follows: less than 30—subnormal; 30 to 60—normal; 60 to 100—mild to moderate; and greater than 100—moderate to severe. The employment of even such crude numerical assessments as guides in deciding the need for splenectomy will assuredly require many case studies. However, Korst, Clatanoff, and Schilling (25) have studied, with methods resembling ours, 3 patients with acquired hemolytic anemia, on whom splenectomies were later performed. In 2 of the patients high splenic accumulations of Cr^{51} occurred and splenectomy was beneficial; in 1 patient, in whom there was little build-up of radioactivity over the spleen, removal of the spleen was ineffectual. There are, in addition to this category of patients, other conditions sometimes characterized by splenomegaly (23, 26–28) in which, although splenectomy is usually not indicated, in individual instances removal of the spleen has benefitted the patient by decreasing the rate of red cell destruction (26–28). An unexpected example eventuated from the observation that radioactivity accumulated in the enlarged spleen of a patient, Case 5, with paroxysmal nocturnal hemoglobinuria following injection of Cr^{51}-labelled red cells. Removal of the patient's 660-gram spleen produced temporarily a moderate diminution of the anemia, and thereafter the patient has required fewer transfusions to maintain a given hemoglobin concentration. It is likely that detectable instances of excessive sequestration by pathologic spleens will arise in a variety of disease states. The surprising predilection of Cr^{51}-labelled pernicious anemia red cells for sequestration by the spleen, observed in Case 8, may explain the therapeutic value of splenectomy reported in this disease prior to the use of liver extract (29). The fact that the defectively formed red cells characteristic of certain diseases of red cell underproduction may survive poorly, and, as in the case of pernicious anemia, despite a calculated rate of hemoglobin production that was subnormal, may be detectably sequestered by the spleen, enjoins the need to characterize the fundamental balance between red cell production and destruction in each case as clearly as possible. The anemia of chronic liver disease, for example, has been shown to be primarily hemolytic in most instances (14). The finding that all of 4 patients with the anemia of liver disease demonstrated mild to moderate splenic uptakes of Cr^{51}, as illustrated by Case 7, indicates the potential therapeutic value of splenectomy in such patients although only rarely is such an operation warranted. The occasional patients with congenital non-spherocytic hemolytic anemia who may benefit from splenectomy should also be identifiable by the Cr^{51} distribution technique.

SUMMARY

The sites of deposition of Cr^{51} in the human body were determined by body surface counting following the injection into normal subjects of Cr^{51}-labelled red cells, hemoglobin, and chromic chloride in saline. Differences characteristic of each were observed and served as a basis for interpretation of a study of a series of 11 patients with hemolytic anemias of diverse kinds. Measurements made on these patients following injection of Cr^{51}-labelled autologous red cells suggested that progressive accumulation of Cr^{51} in the spleen accompanying the disappearance of Cr^{51}-labelled red cells from the blood stream indicates active red cell sequestration. In determining the extent of this sequestration a simple expression may be employed (index of sequestration) which deducts that radioactivity initially present and due simply to the size of the splenic vascular bed from later values obtained over that organ. The methods described should in practice constitute a simple clinical device for determining the need for splenectomy. In certain hemolytic anemias the short survival of labelled red cells in the circulation is accompanied by a progressive increase of radioactivity over the spleen and the anemia is relieved by splenectomy.

ACKNOWLEDGMENTS

We are indebted to Miss Martha Hoge, Miss Patricia Lambert, Miss Nancy Sheldon, and Miss Ann Tomlinson for technical assistance.

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RENAL PARTICIPATION IN ENHANCED PRESSOR RESPONSES TO NORADRENALINE IN PATIENTS GIVEN HEXAMETHONIUM

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(Submitted for publication January 9, 1956; accepted April 16, 1956)

Ganglion-blockers, such as tetra-ethylammonium (TEA) and hexamethonium, enhance responsiveness to vasoactive agents, both pressor and depressor (1). In dogs, the renal arterial bed seems to contribute to the change in blood pressure response since, after giving TEA, adrenaline causes a larger increase and isopropylnoradrenaline (Isuprel®) a larger decrease in renal vascular resistance than in the untreated state (2). The present study was undertaken with the aim of determining responsiveness to noradrenaline in hypertensive patients given hexamethonium and the participation in this of the renal circulation.

PROCEDURES

Brachial auscultatory arterial pressures and plasma clearances of p-aminohippurate (PAH) and mannitol (procedure described in Reference 3) were measured in successive 10-minute periods, under control and test conditions, in the supine position. All the patients had severe hypertensive disease but none of them had signs of congestive heart failure at the time of study. Signs of failure, present on admission in 3 of the patients, had been cleared by diet, digitalis and diuretics for at least a week before test.

Four series of studies were done. In the first (I), 6 patients, were observed as below during a 30-minute control period interval; they were then given noradrenaline by infusion at rates of 4 or 5 micrograms per minute over about 40 minutes, during the last 30 of which 3 urine collections were made, the urine formed during the first 10 minutes having been discarded; single intravenous injections of hexamethonium were then given in doses sufficient to decrease diastolic pressure about 10 to 20 mm. Hg and the effects of this observed over another 40 minutes in the same way as had been those of noradrenaline; at the end of this time noradrenaline was again infused in the same dose as before and the observations repeated. Blood pressures (measured at intervals of 2 to 5 minutes), plasma clearances and rates of urine flow were averaged for each of the four sets of three 10-minute urine collection periods.

In the second series (II), 4 patients were given noradrenaline after control observations as above. In the third series (III), 6 patients were given hexamethonium after control study and, in the fourth (IV), 7 patients under chronic treatment with hexamethonium were given noradrenaline as above.

The plasma clearance of PAH at low plasma concentrations (C_{PAH}) was taken as equivalent to renal plasma flow (RPF) and the plasma clearance of mannitol times 1.1 (to allow for 10 per cent reabsorption) was taken as equal to glomerular filtration rate (GFR). Urine minute volume (V) was estimated from the excess over that of the 100 ml. of NaCl used to rinse the bladder. Renal vascular resistance (R) was calculated as Pm-5/RPF, taking as Pm (mean arterial pressure) the mean of the averages of systolic and diastolic pressure over the respective minute interval of study.

RESULTS

The observations are compiled in Tables I and II. Table III condenses the data by listing the average magnitudes of these effects as ratios of test to control levels.

Noradrenaline alone

Series I and II. This caused a light increase in arterial pressure in 9 of the 10 patients. It increased renal vascular resistance in all, this proportionately more than it did arterial pressure. It elicited increases in urine flow in 7 of the 10 tests.

Hexamethonium alone—single injection

Series I and III. This agent, in the widely varying doses used to elicit the desired moderate depressor effects, had irregular effects on renal resistance, which was increased in 3 and decreased in 8 of the 12 tests with no change in 1. Renal plasma flow was decreased to some extent in 10 of the 12 tests; glomerular filtration rate and urine flow were decreased in all.

The association between changes in filtration

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TABLE I

Effects of noradrenaline, hexamethonium and of noradrenaline in the presence of hexamethonium *

No.	Diag.	RPF	GFR	FF	B.P. mm. Hg	R	V	Treatment
1	EHM	198	40	.20	225/121	.84	1.2	None
		188	39	.21	225/126	.93	2.3	N-A 4μ per min.
		194	32	.17	199/115	.78	0.4	Hexam. 75 mg.
		213	34	.16	214/128	.78	0.8	N-A 4μ per min.
3	EHM	414	108	.26	211/118	.39	4.8	None
		365	112	.21	237/122	.48	7.2	N-A 4μ per min.
		398	102	.26	183/104	.32	2.0	Hexam. 50 mg.
		386	101	.26	218/120	.42	4.5	N-A 4μ per min.
4	EHM	465	117	.25	213/136	.37	8.3	None
		336	99	.29	236/148	.56	6.3	N-A 4μ per min.
		344	88	.28	190/129	.45	1.7	Hexam. 5 mg.
		359	105	.29	225/148	.51	4.5	N-A 4μ per min.
5	EH	415	112	.27	194/106	.36	4.0	None
		353	99	.28	205/112	.43	4.6	N-A 4μ per min.
		318	72	.23	166/96	.41	0.75	Hexam. 10 mg.
		334	83	.25	180/106	.41	0.8	N-A 4μ per min.
7	EHM	169	38	.22	185/120	.89	2.3	None
		142	30	.21	204/125	1.13	2.7	N-A 5μ per min.
		140	26	.19	120/85	.69	0.25	Hexam. 15 mg.
		133	29	.22	146/100	.89	0.3	N-A 5μ per min.
9	EHS	251	68	.27	185/106	.56	8.5	None
		201	55	.27	195/110	.74	6.0	N-A 5μ per min.
		170	47	.28	134/93	.64	0.1†	Hexam. 16 mg.
		235	50	.21	182/120	.64	2.4	N-A 5μ per min.

* Renal effects of noradrenaline infusion (N-A) and hexamethonium administration (Hexam.) and of noradrenaline infusion after hexamethonium as measured from renal plasma flow (RPF = plasma clearance of PAH), and glomerular filtration rate both in ml. per min. per 1.73 sq. m. of body surface (GFR = plasma mannitol clearance times 1.1), filtration fraction (FF = GFR/RPF) and averages of arterial pressure (Pm = systolic + diastolic/2), renal vascular resistance ($R = Pm\text{-}5/C_{PAH}$), and urine volume, ml. per min. (V) in 6 patients with severe (S) or malignant (M) essential hypertension (EH).

† Estimated maximum of urine flow during oliguria.

rate and urine flow in these experiments is shown in Figure 1A. This shows that, during the 40 to 50-minute interval after a single injection of hexamethonium, the antidiuresis which occurred was consistently associated with decreased glomerular filtration. With one exception, the changes in urine flow occurred in rough proportion to changes in filtration rate and small decreases in filtration elicited large decreases in urine flow. The effects resemble those described in previous reports (4–7).

Pressor effect of noradrenaline during the action of hexamethonium (a)

Series I. Single injections of hexamethonium decreased average blood pressure by about 20 per cent. Subsequent infusion of noradrenaline at rates of 4 and 5 micrograms per minute caused consistent rises of blood pressure which averaged about 20 per cent above the post-hexamethonium level. Thus, the absolute levels of pressure attained during these infusions of noradrenaline were about the same as existed during the pre-hexamethonium noradrenaline infusions, but the mm. Hg rise of pressure was much greater.

Series IV. Patients under chronic treatment with hexamethonium also showed increased pressor responsiveness. The mean increase of pressure average in this series was about 30 per cent. Since preinfusion pressure levels of many of these patients were already high, the levels reached during noradrenaline infusion were sometimes very high and greatly exceeded those reached in patients who had been given only single doses of hexamethonium.

Renal effects of noradrenaline during the action of hexamethonium

Series I and IV. Noradrenaline had like renal effects in both hexamethonium-treated series. The average of the changes in renal vascular re-

sistance indicates an increase; the response of this function, unlike that of blood pressure, was not augmented by hexamethonium. Rather, the responses were unexpectedly variable, with no significant changes in renal resistance in 3 of the 6 patients given single injections of hexamethonium and in 2 of the 7 under chronic treatment. These variable responses of renal resistance to nor-

TABLE II

Effects of noradrenaline and hexamethonium in hypertensive patients and noradrenaline in patients under chronic treatment with hexamethonium *

No.	Diag.	RPF	GFR	FF	B.P.	R	V	Dose
				A. Noradrenaline				
10A	EHM	317	80	.25	224/134	.55	7.8	
		275	79	.29	248/135	.69	6.7	N-A 5μ per min.
17	EHM	298	84	.28	189/115	.48	2.6	
		264	78	.30	192/110	.54	3.9	N-A 5μ per min.
19	EHS	362	92	.23	200/115	.42	6.1	
		287	79	.27	212/124	.58	8.4	N-A 4μ per min.
20	EHS	290	63	.22	190/122	.53	0.9	
		289	65	.22	216/140	.60	2.6	N-A 5μ per min.
				B. Hexamethonium				
22A	EHM	81	30	.27	224/134	2.19	3.6	
		71	21	.30	158/196	1.75	1.2	Hexam. 26 mg.
22B	EHM	96	31	.32	205/120	1.55	6.8	
		104	23	.22	188/144	1.40	1.5	Hexam. 20 mg.
23	EHM	176	62	.35	210/136	1.00	2.6	
		138	35	.25	200/138	0.88	0.75	Hexam. 13 mg.
24	EHS	324	72	.22	230/120	.51	4.8	
		330	65	.20	160/97	.38	0.5	Hexam. 13 mg.
25	EHS	312	82	.26	200/144	.53	1.7	
		322	76	.24	168/130	.44	0.6	Hexam. 36 mg.
26	EHM	234	50	.22	185/115	.62	2.2	
		174	32	.18	137/90	.62	0.6	Hexam. 28 mg.
				C. Noradrenaline during chronic hexamethonium treatment				
2	EHM	109	33	.30	196/120	1.39	3.1	
		91	30	.33	260/147	2.19	4.6	N-A 4μ per min.
8	EHM	159	43	.27	200/140	1.04	5	
		159	50	.31	278/170	1.37	12.4	N-A 5μ per min.
10B	EHM	293	62	.21	185/106	0.48	0.4	
		324	75	.23	250/135	0.58	3.3	N-A 5μ per min.
13	EHM	240	67	.28	210/140	.70	5.6	
		270	73	.27	232/146	.68	5.6	N-A 5μ per min.
14B	EHM	263	46	.28	194/128	.96	5.5	
		167	48	.29	216/137	1.03	8.8	N-A 4μ per min.
16	EHS	363	99	.27	174/118	0.39	7.7	
		369	119	.32	230/134	.49	17	N-S 4.6μ per min.
1	EHM	259	43	.17	158/110	.49	0.8	
		244	55	.23	264/153	.83	10.7	N-A 4μ per min.

* Conventions as in Table I.

TABLE III

Summary of observations *

	A. Noradrenaline, 10 cases					B. Hexamethonium, 12 cases				
	Pm	RPF	GFR	R	V	Pm	RPF	GFR	R	V
Mean	1.07	.88	.91	1.25	1.39	.85	.84	.77	.93	.25
Range	1.0–1.13	.7–1.0	.8–1.0	1.1–1.5	.7–2.9	.7–.9	.6–1.1	.6–.93	.8–1.2	.1–.4

Noradrenaline after Hexamethonium

	C. Recent injection, 6 cases					D. Chronic treatment, 7 cases				
	Pm	RPF	GFR	R	V	Pm	RPF	GFR	R	V
Mean	1.19	1.08	1.09	1.11	2.0	1.29	1.00	1.13	1.30	4.3
Range	1.1–1.4	.9–1.4	1.0–1.2	1.0–1.3	1.2–2.6	1.1–1.6	.8–1.1	.9–1.3	1.0–1.7	1.0–1.3

* Means of ratios of functions listed as Tables I and II. Ratios: (A) noradrenaline to control data and (B) hexamethonium to control data; (C) noradrenaline to status elicited by a single injection of hexamethonium and (D) to that existing during chronic hexamethonium treatment.

adrenaline infusion in the face of consistent, augmented increases of arterial pressure resulted from the varied changes in C_{PAH}, which was increased in 6 of the 13 tests, *viz.*, in 4 instances of series I and 2 of series IV.

Glomerular filtration was increased by noradrenaline infusion in 7 of the 13 tests, on the average by about 10 per cent. Urine flow increased in 10 of these tests.

Noradrenaline diuresis

The diuretic effect of noradrenaline (Figure 1B) had no relation to pre-treatment with hexamethonium. Urine flows increased in patients whose filtration rates were not altered by noradrenaline infusion and in those whose filtration rates were somewhat increased. Small increases in urine flow were also observed in 3 patients whose rates of glomerular filtration were depressed during noradrenaline infusion by about 10 per cent, but in another 3 whose glomerular filtration rates were decreased 10 per cent or more, urine flow was also depressed.

DISCUSSION

Enhancement of pressor responsiveness to noradrenaline after treatment with hexamethonium is an instance of the general increased responsiveness

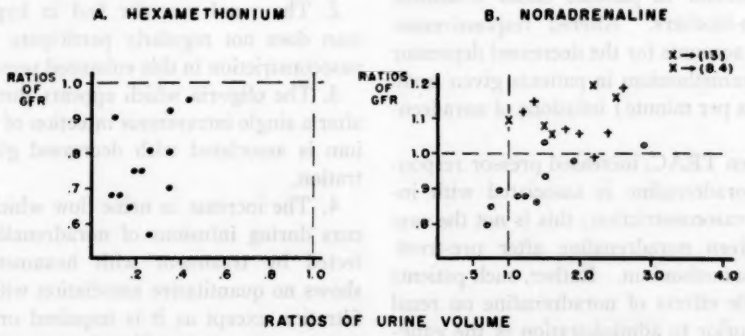

FIG. 1. ORDINATES: RATIOS OF GLOMERULAR FILTRATION RATES (GFR) IN CONTROL AS COMPARED WITH TEST PERIODS. ABSCISSAE: RATIOS OF AVERAGE URINE VOLUME IN CONTROL/TEST PERIODS

A. Control: No treatment; tests, single injections of hexamethonium. B. Control: No pre-treatment (open circles), after single injections of hexamethonium (crosses) or during chronic treatment with hexamethonium (x's): tests were infusion of noradrenaline.

of blood pressure to vasoactive agents which is elicited by ganglion-blocking drugs (1). If it is assumed that the vasomotor center cannot homeostatically moderate changes in arterial pressure by reciprocal adjustments of vasomotor tone, this is a predictable response.

The increase in responsiveness to noradrenaline is greater, both in mm. Hg pressure rise and in the final levels reached, in patients under chronic treatment than in those given only single injections of hexamethonium. This may be only a dosage effect, since it can be presumed that the effective body store of ganglion-blocker is greater in patients under continuous chronic treatment than in those given only single, moderately depressor doses. Alternatively, the difference may depend on the state of the arterial tree in these two groups of patients; in those given single injections, there is abrupt partial loss of vasomotor tone with resultant vasodilation, while most of the patients under chronic treatment had presumably regained their vasoconstricted state with the return of supine blood pressure to hypertensive levels. The difference in this case would result from the greater changes in peripheral resistance and blood pressure which should be elicited by equal degrees of vasoconstriction in a vasodilated as compared with a vasoconstricted vascular bed. Whatever the mechanism, the experiments confirm the suggestion (8) that doses of pressor agents which are only slightly effective under normal conditions may be severely pressor in patients under treatment with ganglion-blockers. Altered responsiveness doubtless also accounts for the decreased depressor activity of hexamethonium in patients given small (2 micrograms per minute) infusions of noradrenaline (9).

In dogs given TEAC, increased pressor responsiveness to noradrenaline is associated with increased renal vasoconstriction; this is not the case in patients given noradrenaline after pre-treatment with hexamethonium. Rather, such patients sustain variable effects of noradrenaline on renal plasma flow; prior to administration of the ganglion-blocker, noradrenaline consistently elicits renal vasoconstriction with little change in arterial pressure; after hexamethonium has been given, noradrenaline has irregular effects on renal plasma flow and fails to elicit increases in renal vascular resistance in a third of the tests, although blood pressures are consistently and sometimes severely increased.

The nature of this seeming failure of the renal vascular bed to participate in a pressor response attributable primarily to vasoconstriction is not clear. One consideration is that vascular disease may impair the 'autonomy of the renal circulation' of some patients with hypertensive disease. Such impairment would account for the renal bed sometimes responding as a passive system as it may during hypotension caused by spinal anesthesia (10) or, as in these experiments, during abrupt, large rises of blood pressure. Such passive responses are observed even in normal animals, such as in dogs brought into hypotension by bleeding, in which noradrenaline in amounts which return blood pressure to normal does not elicit consistent increases in renal vascular resistance (11).

Finally, the data indicate that noradrenaline-induced increases of urine flow are not proportionate to concurrent changes in filtration rate, except as the diuretic response is impaired or reversed by decreased rates of glomerular filtration; noradrenaline diuresis is not affected by hexamethonium.

SUMMARY AND CONCLUSION

1. The pressor effect of infused noradrenaline is enhanced in hypertensive patients given single injection or under chronic treatment with hexamethonium.

2. The renal vascular bed in hypertension in man does not regularly participate by increased vasoconstriction in this enhanced pressor response.

3. The oliguria which appears during the hour after a single intravenous injection of hexamethonium is associated with decreased glomerular filtration.

4. The increase in urine flow which usually occurs during infusions of noradrenaline is not affected by treatment with hexamethonium and shows no quantitative association with glomerular filtration except as it is impaired or reversed by decreased glomerular filtration.

Addendum

Since this manuscript was submitted, our attention has been drawn to the paper on "Reactivity to Pressor Agents in Hypertension" (Circulation, 1955, 12, 974) by A. E. Doyle and H. Black, in which they show that hy-

pertensive patients yield larger pressor responses to angiotonin, S-methyl iso-thiourea and noradrenaline after hexamethonium than before and also larger increases in these responses than those in normotensive patients similarly tested.

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THE EFFECT OF THYROID DISEASE ON CALCIUM METABOLISM IN MAN[1]

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(Submitted for publication October 31, 1955; accepted April 24, 1956)

Abnormal thyroid function profoundly alters calcium metabolism. In 1929, Aub, Bauer, Heath, and Ropes (1) demonstrated in patients on constant low calcium diets that urinary and fecal excretion of calcium and phosphorus is frequently increased in hyperthyroidism and lower than normal in myxedema. These changes could not be ascribed to elevation of metabolism *per se* (1) acidosis (2) or vitamin D deficiency (3); they were observed even in the absence of the parathyroid glands (4, 5). In addition, roentgenographic evidence of skeletal demineralization has been described in patients with thyrotoxicosis (6).

The conventional balance techniques which have been used in the study of calcium metabolism in thyroid disease measure only net effects: they do not measure the processes of deposition and resorption of calcium. An indication of the skeletal turnover of calcium, however, can be obtained from serial observations of the specific activity of radioactive calcium (Ca^{45}) in blood and urine after intravenous injection (7, 8).

The present study was designed to define the alterations in calcium metabolism in thyroid disease in man by using Ca^{45} and the technique of isotope dilution. Observations have been made in euthyroid, hyperthyroid, and myxedematous subjects, and, for purposes of comparison, in additional euthyroid patients with Paget's disease and with hypoparathyroidism.

MATERIALS AND METHODS

All patients (Table I) were on the metabolic ward and were ambulatory throughout the study. Diets, which were constant and of neutral ash, contained from 0.08 to 0.23 grams of calcium per day (Table II). In order to avoid tetany, the two hypoparathyroid subjects were given 0.31 and 0.57 grams, respectively, of calcium in their diets per day. Fluid intake was maintained at 2,000 to 2,500 ml. per day. Complete collections of urine and stool were begun after six days on the fixed diet. A three-day control collection of urine and feces was obtained prior to the administration of Ca^{45}.

The stock solution of $Ca^{45}Cl_2$[2] was acidified with concentrated HCl, added to 0.9 per cent NaCl solution to give a final concentration of approximately 0.5 μc per ml. saline at a pH of 6, and autoclaved. Five to seven microcuries were injected from a calibrated syringe into an antecubital vein one hour before breakfast. Blood samples were obtained by venipuncture from the opposite arm at frequent intervals. Fractional urine collections were made for the first three days following injection, and daily thereafter. Stool collections were made

TABLE I

Clinical data

Name	Age	Sex	Diagnosis
R. P.	50	M	Coronary heart disease; hyperuricemia (A.S.H.D.)*
Wa. W.	26	M	Friedreich's ataxia (Fr.A.)
F. W. (1)	18	M	Hyperthyroid (diffuse goiter) (Thyrtox.)
(2)	19		Euthyroid, hypoparathyroid, 1 yr. following thyroidectomy (Hypoparathyr. after Rx.)
L. S. (1)	31	F	Hyperthyroid (diffuse goiter) (Thyrtox.)
(2)	31		Early myxedema (4 months following thyroidectomy) (Myx. after Rx.)
C. L.	38	F	Hyperthyroid (diffuse goiter) (Thyrtox.)
J. B.	26	M	Hyperthyroid (diffuse goiter) (Thyrtox.)
W. W. (1)	42	F	Myxedema (Myx.)
(2)	42		Euthyroid (4 months on treatment) (Normal after Rx.)
B. M. (1)	39	F	Myxedema (Myx.)
(2)	39		Euthyroid (5 months on treatment) (Normal after Rx.)
A. W.	67	F	Paget's disease of bone (Osteitis Deformans) (Paget's dis.)
O. H.	59	F	Paget's disease of bone (Osteitis Deformans) (Paget's dis.)
E. N.	41	F	Hypoparathyroid, 4 years following thyroidectomy (Hypoparathyr.)

* Abbreviations to be used in all tables.

[1] Aided by grants AT (30-1) 667 and AT (30-1) 1755 from the Atomic Energy Commission and A(446)C from the United States Public Health Service.

[2] Obtained from Oak Ridge. Ca^{45} has only a beta ray (M.E.V. = 0.26) and a half-life of 152 days.

TABLE II

Metabolic balance data and serum values

Patient	Number of 3-day periods averaged	Calcium (*Gm./day*)			Phosphorus (*Gm./day*)			Nitrogen (*Gm./day*)			Body weight (*Kg.*)	Serum				Basal metabolic rate (%)
		Urine	Feces	Intake	Urine	Feces	Intake	Urine	Feces	Intake		Calcium (*Mg. %*)	Phosphorus (*Mg. %*)	Alk. P'tse. (*Bodansky units %*)	Protein bound iodide (*Microg. %*)	
R. P. (A.S.H.D.)	8	0.062*	0.110	0.148	0.469	0.264	0.743	10.678	1.437	13.920	74.6	9.7	3.8	3.6	3.5	
Wa. W. (Fr.A.)	4	0.158	0.175	0.123	0.545	0.239	0.857	10.234	1.169	11.984	56.0	10.1	4.0	2.4		
F. W. (1) (Thyrtox.)	5	0.747	0.597	0.232	1.595	0.651	1.448	19.860	3.471	22.280	57.9	10.3	4.7	10.2	21.1	+40
F. W. (2) (Hypoparathyr. after Rx)	4	0.016	0.176	0.314	1.018	0.367	1.782	20.910	2.475	29.440	71.8	6.6	6.6	4.7	5.2	+ 5
L. S. (1) (Thyrtox.)	4	0.118	0.108	0.076	0.750	0.516	1.060	10.862	1.768	11.480	54.5	9.3	3.9	4.0	14.4	+35
L. S. (2) (Myx. after Rx)	4	0.018	0.062	0.082	0.347	0.259	0.688	7.213	1.912	10.120	60.3	8.8	3.5	3.4	0.5	−28
C. L. (Thyrtox.)	4	0.150	0.264	0.092	0.695	0.249	0.704	10.208	1.274	10.640	52.9	10.0	4.3	2.5	13.2	+39
J. B. (Thyrtox.)	4	0.331	0.306	0.200	1.242	0.309	1.240	17.061	2.106	19.200	69.7	9.7	4.5	4.1	9.5	+24
W. W. (1) (Myx.)	8	0.053	0.137	0.104	0.502	0.268	0.740	7.071	1.278	10.440	64.0	9.1	3.9	1.5	0.6	−40
W. W. (2) (Normal after Rx)	6	0.124	0.162	0.093	0.642	0.176	0.695	8.889	0.986	10.080	54.3	9.4	4.6	1.6		−10
B. M. (1) (Myx.)	8	0.038	0.129	0.110	0.369	0.222	0.528	7.793	1.095	10.600	63.7	9.6	3.5	1.2	1.2	−48
B. M. (2) (Normal after Rx)	8	0.126	0.216	0.084	0.588	0.177	0.570	9.981	1.039	11.200	56.9	10.0	3.8	1.9		−12
A. W. (Paget's disease)	7	0.129	0.068	0.120	0.403	0.167	0.562	7.288	1.176	8.680	43.7	9.6	3.7	58.5		
O. H. (Paget's disease)	4	0.208	0.154	0.122	0.788	0.231	0.560	8.822	1.100	11.360	54.3	9.9	4.3	33.9	3.9	
E. N. (Hypoparathyr.)	4	0.022	0.615	0.566	0.494	0.371	0.668	10.056	1.189	11.000	66.6	6.2	6.1	0.8		+13

* Mean value.

in three-day periods. An aliquot of the solution used for injection was taken for the standard. The amount of stable calcium (Ca^{40}) included in each injection ranged from 1 to 6 mgm. After giving the Ca^{45}, collections were made for times ranging from 9 to 22 days for the various patients. If a previous study had been done, control collections were assayed for radioactivity prior to the administration of the second tracer of Ca^{45}.

Stable calcium (Ca^{40}) was determined by the method of Fiske and Logan (9). The results of analysis of duplicate samples agreed within ± 3 per cent of the mean. Phosphorus [3] was determined by the method of Fiske and Subbarrow (10) and nitrogen according to the macromethod of Kjeldahl. Aliquots of urine (200 ml.) were acidified with 4 ml. of concentrated HCl. The 3-day stool collections (obtained without a marker) were mixed in a Waring Blendor, and made up to volume with distilled water. For calcium and phosphorus analyses, duplicate 200-ml. aliquots of the blended and diluted fecal samples were dried for 2 to 3 days in a steam oven and then ashed in an electric muffle furnace at 550° C. overnight. The ash was dissolved in concentrated HCl and distilled water. Nitrogen analyses of the feces were performed directly on unashed aliquots of the blended and diluted fecal samples.

Ca^{45} was determined as follows: [4] 4 ml. of trichloracetic acid (20 per cent) and 8 ml. of distilled water were added to 2.4 ml. of serum, the sample centrifuged, and the Ca^{45} determination performed on the supernatant. Complete recovery of Ca^{45} added to human serum *in vitro* was achieved. Analyses of Ca^{45} in urine and feces were made on acidified specimens which had been dried and ashed, and the ash dissolved in HCl and distilled water. After analysis for the stable calcium of the sample, stable calcium was added as $CaCl_2$ to give a final concentration of 6.0 mgm. per sample in all specimens. From a self-absorption curve this concentration was found to be optimal for sensitivity and reproducibility of sample measurements. Calcium was then precipitated as calcium oxalate by the addition of saturated ammonium oxalate solution. The solution was made alkaline by the addition of dilute ammonium hydroxide and then adjusted to a pH of 5.4 with 2.5 per cent oxalic acid, using an alcoholic solution of methyl red as indicator. After adding Triton X-100 [5] (0.1 per cent) as a detergent, the samples were filtered over sintered glass on filter paper discs, 24 mm. diameter (Schleicher and Schuell No. 576), which were cemented to copper discs with polystyrene coil dope.

The discs from initial studies were assayed for Ca^{45} under a thin (< 1.9 mgm. per cm.^{2}) mica end-window G.M. tube connected to an automatic sample changer (Tracerlab). In later studies, a gas-flow counter was used which increased sensitivity from 250,000 to 500,000 counts per minute per microcurie. Each of the duplicate samples was measured three times to an accumulation of 4,096 counts. The standard was assayed with each run. The maximum variation in accumulated counts of duplicate samples was ± 6 per cent of the mean. The usual variation was ± 3 per cent. Recorded radioactivity of the samples was at least twice background.

The data for Ca^{45} are expressed as per cent administered dose. Specific activity, as per cent of administered dose of labeled calcium per gram of calcium, was calculated for each sample.[6]

METHODS OF ANALYSIS

Inspection of the semi-logarithmic plot of specific activities in urine and serum against time suggested that the curves could be resolved into a series of three or possibly more decreasing exponential functions. This was of interest because of the possibility that each function represented a discrete calcium compartment within the body. The data were treated with this assumption in view.

Analysis of exponents: The method of curve analysis is illustrated in Figure 1 where serum and urine specific activities of an hyperthyroid subject are plotted against time in days. Serum specific activities are plotted for the first day. Since the radioactivity of many serum samples was low by 24 hours following the administration of Ca^{45}, urine specific activities are plotted from the second day onward. The data of all subjects have been treated in an identical manner. In Figure 1 (A) are plotted the observed specific activities from 0 to 9 days. The data from 1.25 to 8.5 days appeared to follow a single exponential function. By the method of least squares a fit of this range of the data to the function $A_3e^{-k_3t}$ yielded the solid line of Figure 1 (A) (A_3 is specific activity extrapolated to zero time, k_3 is the rate constant in fraction per day, and t is time in days). When this function was subtracted from the observed data, the values in Figure 1 (B) were derived. An analysis by least squares of the data between 0.13 and 0.67 days yielded the solid line in Figure 1 (B). When this line of slope k_2 was extrapolated to zero time, the specific activity value of A_2 was obtained. This line subtracted from the remaining data of Figure 1 (B) yielded the points in Figure 1 (C). The solid line in Figure 1 (C) is the least squares fit of those data with slope k_1, and when extrapolated to zero time gives A_1. Thus, the data are analyzed in the form:

$$\text{Specific activity} = A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t}.$$

Compartments: The size of each "compartment" (Q) was obtained by dividing 100 per cent (the quantity of labeled calcium at zero time) by the extrapolated specific

[3] The authors are indebted to the hospital chemistry laboratory under Dr. Charles Dutoit and Miss G. Margaret Rourke for the phosphorus and nitrogen analyses.

[4] This method was developed by Dr. C. J. Maletskos of the Massachusetts Institute of Technology.

[5] Obtained from Rohm and Haas Co., Philadelphia 5, Pennsylvania.

[6] The term "labeled calcium" refers to that quantity of calcium which was initially given to the patient as a tracer dose marked with radioactive calcium. This quantity is independent of radioactive decay.

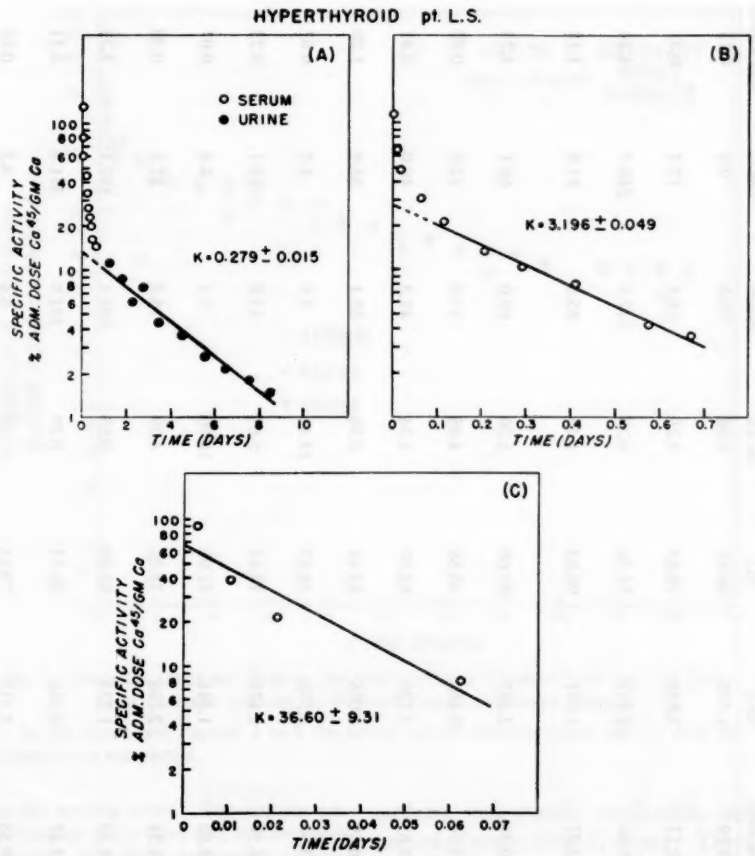

FIG. 1. ILLUSTRATION OF THE METHOD OF ANALYSIS OF EXPONENTIAL FUNCTIONS OF SPECIFIC ACTIVITY CURVE PLOTTED SEMI-LOGARITHMICALLY VERSUS TIME

The data obtained from subject L. S.(1) (hyperthyroid) are used as examples. Note the different time scale for (A), (B) and (C). The specific activity values on the ordinate are the same for (A), (B) and (C). The solid line in (A) has the slope k_3 which extrapolated to zero time yields A_3; the solid line in (B) has the slope k_2 and intercept A_2; the solid line in (C) has the slope k_1 and intercept A_1. For further explanation, see the text.

activity value (A) for each function. For the third compartment (Q_3) this equals $\frac{100\%}{A_3}$; for the second (Q_2), $\frac{100\%}{A_2 + A_3}$; for the first (Q_1), $\frac{100\%}{A_3 + A_2 + A_1}$. This analysis is rigorously correct only when the size of the compartments (Q) increases and the flow (kQ) decreases from one to the next compartment.

Calcium "pool": If specific activity

$$\left(i.e., \frac{\%\text{ administered dose } Ca^{45}}{\text{gram } Ca^{40}}\right)$$

is known at time t, and the quantity retained in the body (100 per cent of administered Ca^{45} minus per cent excreted) is known, the per cent retained at time t divided by the specific activity at time t is the calcium "pool" at time t. The concept of a calcium "pool" makes no assumption based on the shape of the curves, but reflects the fact that a lower specific activity at any time means a larger mass of dilution. Calcium "pool" curves were derived from the ratio between the curve of labeled calcium retention and the specific activity curve for the various patients.

The analysis of calcium metabolism presented here assumes that the calcium is first present in a small compartment with a rapid flow to and from a larger second compartment. This flow accounts for the initial rapid

TABLE III

Excretion data and calcium "pool" at nine days

Patient	Total Ca^{45} 0–9 days (urine) (% *adm. dose*)	Total Ca^{45} 0–9 days (urine) (*Gm.*)	Total Ca^{45} 0–9 days (urine and feces) (% *adm. dose*)	Total Ca^{45} 0–9 days (urine and feces) (*Gm.*)	Ca^{45} retained at 9 days (%)	Ca^{45} spec. act., 8.5 days (% *adm. dose/ Gm. Ca*)	Ca pool (*Gm.**)	Adjusted Ca pool (*Gm.*†)	Adjusted Ca pool/ body weight (*Gm./Kg. body weight*)
R. P. (A.S.H.D.)	6.26	0.474	13.19	1.595	86.81	8.06	10.8	9.9	0.13
Wa. W. (Fr.A.)	12.85	1.372	21.17	2.834	78.83	4.26	18.5	17.2	0.31
F. W. (1) (Thyrtox.)	17.70	7.334	25.66	12.727	74.34	0.30	247.8	245.7	4.24
F. W. (2) (Hypoparathyr. after Rx.)	0.66	0.149	3.37	1.627	96.63	1.13	85.5	84.6	1.18
L. S. (1) (Thyrtox.)	6.71	1.138	10.34	2.103	89.66	1.30	69.0	68.1	1.25
L. S. (2) (Myx. after Rx.)	1.34	0.152	3.44	0.460	96.56	4.05	23.8	22.9	0.38
C. L. (Thyrtox.)	8.18	1.413	17.63	3.730	82.37	1.74	47.3	46.0	0.87
J. B. (Thyrtox.)	10.88	3.036	16.59	6.005	83.41	0.98	85.1	83.6	1.20
W. W. (1) (Myx.)	9.10	0.432	23.54	1.590	76.45	15.40	5.0	4.2	0.07
W. W. (2) (Norm. after Rx.)	14.27	1.062	26.59	2.475	73.41	5.65	13.0	12.1	0.22
B. M. (1) (Myx.)	7.01	0.351	18.02	1.341	81.98	16.00	5.1	4.4	0.07
B. M. (2) (Norm. after Rx.)	11.94	1.045	21.74	2.760	78.26	4.29	18.2	17.2	0.30
A. W. (Paget's dis.)	3.07	1.125	4.10	1.723	95.90	0.93	103.1	102.1	2.33
O. H. (Paget's dis.)	2.21	1.889	3.47	3.284	96.53	0.29	332.9	331.2	6.11
E. N. (Hypoparathyr.)	4.71	0.200	29.55	5.547	70.45	16.20	4.4	3.5	0.05

* Calculated: $\text{Ca Pool} = \dfrac{\%\ Ca^{45}\ \text{Remaining at 9 days}}{\text{Urine Spec. Act. (\% Adm. Dose/Gm.) at Day 8.5}}$.

† Calculated: $\text{Adjusted Ca Pool} = \dfrac{\%\ Ca^{45}\ \text{Remaining at 9 days} - \%\ Ca^{45}\ \text{in ECF at 9 days}}{\text{Urine Spec. Act. (\% Adm. Dose/Gm.) at Day 8.5}}$.

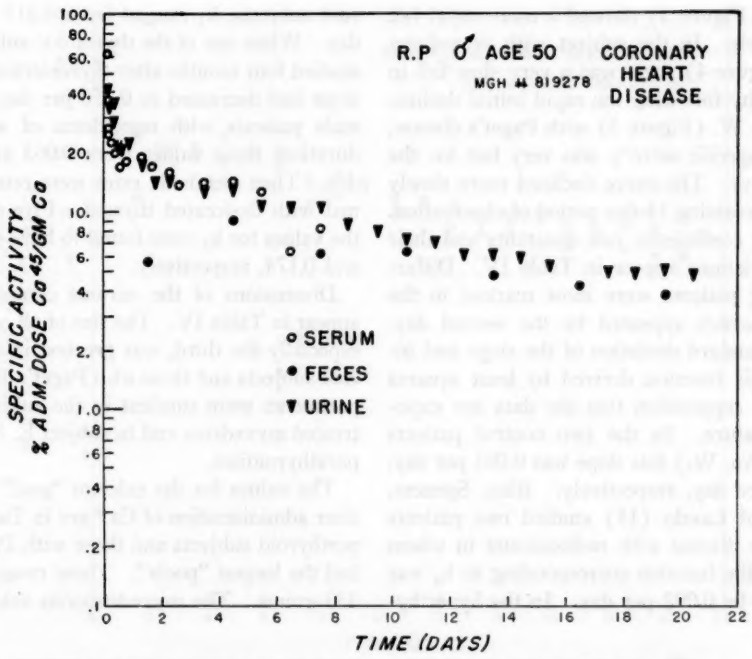

FIG. 2. SEMI-LOGARITHMIC PLOT OF SPECIFIC ACTIVITY VERSUS TIME FOR THE DATA FROM CONTROL SUBJECT R. P. WITH CORONARY HEART DISEASE

In this figure and Figures 3 to 5 the serum specific activity values for the first 30 minutes are not plotted.

decline in the specific activity curve. The analysis continues in the same manner into a third and, in some cases, a fourth compartment. The basic assumption of equal specific activities after equilibrium can be made in an open system (*i.e.*, a system involving excretion of the substance) only when the amount being excreted is small compared to the other routes of flow to and from the compartments being studied. The data appear to validate these assumptions over the time interval studied. However, at longer times, the specific activity of the urine and blood may diverge markedly from that of the bone compartments because the flow from the large bone compartments may be small compared to the ingestion and excretion of unlabeled calcium.

RESULTS

Ca^{45} data

The concentration of labeled calcium in the serum declined rapidly following injection and could not be measured in most patients in the sample taken seven days after administration of the radioactive calcium. Within the first hour after injection, Ca^{45} appeared in the urine. Urine radioactivity was readily measurable throughout the period of study (9 to 21 days). There was no correlation between the total labeled calcium and the total stable calcium excreted over a 9-day period of observation (Table III).

Urine and serum specific activities yielded curves of characteristic form when plotted against time on semi-logarithmic paper (Figures 2–5). The specific activity of calcium in the serum was in all cases the same as that of the urine collection corresponding to the serum sample. Fecal specific activity was almost always less than that of the urine or serum sample in the midpoint of the collection period and fell off at a rate similar to that of urine.

In Figure 2 are plotted the data of a control subject (R. P.). An initial rapid fall in specific activity of blood and urine was followed by a slower, linear decline from the second to the eighteenth day after administration of Ca^{45}. In contrast, the curve of specific activity of the hyperthyroid sub-

ject (F. W., Figure 3) showed a more rapid fall to lower levels. In the subject with myxedema (W. W., Figure 4) there was a very slow fall in specific activity following the rapid initial decline. In subject A. W. (Figure 5) with Paget's disease, the drop in specific activity was very fast for the first eight days. The curve declined more slowly during the remaining 14-day period of observation.

Values for coefficients, rate constants and their standard deviations appear in Table IV. Differences among patients were most marked in the third slope which appeared by the second day. The small standard deviation of the slope and intercept of this function derived by least squares supports the supposition that the data are exponential in nature. In the two control patients (R. P. and Wa. W.) this slope was 0.081 per day, and 0.164 per day, respectively. Blau, Spencer, Swernov, and Laszlo (11) studied two patients without bone disease with radiocalcium in whom the slope of the function corresponding to k_3 was calculated to be 0.092 per day. In the hyperthyroid subjects, k_3 ranged from 0.215 to 0.288 per day. When one of the thyrotoxic subjects was restudied four months after thyroidectomy, this third slope had decreased to 0.150 per day. In two female patients with myxedema of several years' duration, these values were 0.063 and 0.072 per day. Then metabolic rates were returned to normal with desiccated thyroid. Five months later, the values for k_3 were found to have risen to 0.150 and 0.174, respectively.

Dimensions of the various compartments also appear in Table IV. The size of all compartments, especially the third, was greatest in the hyperthyroid subjects and those with Paget's disease. Compartments were smallest in the subjects with untreated myxedema and in subject E. N. with hypoparathyroidism.

The values for the calcium "pool" at nine days after administration of Ca^{45} are in Table III. Hyperthyroid subjects and those with Paget's disease had the largest "pools". These ranged from 47 to 333 grams. The myxedematous subjects and the

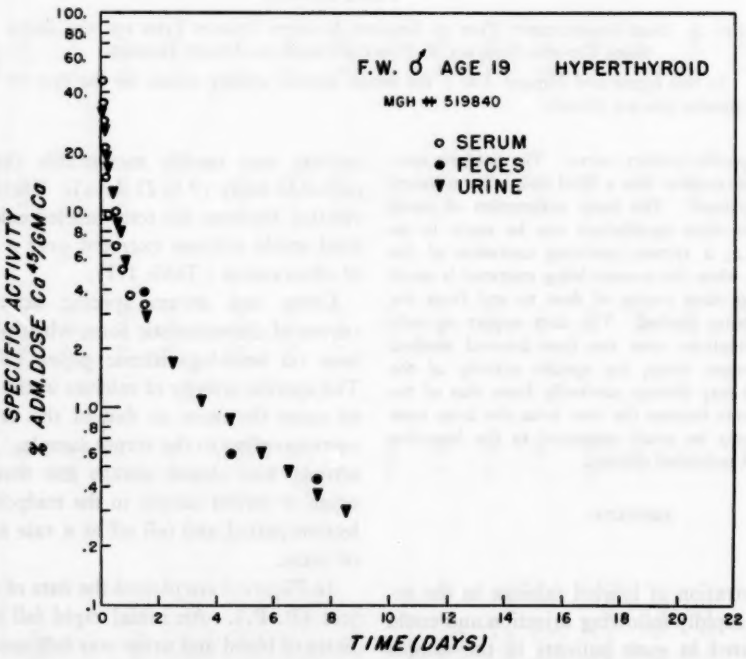

FIG. 3. SEMI-LOGARITHMIC PLOT OF SPECIFIC ACTIVITY VERSUS TIME FOR SUBJECT F. W.(1) WITH HYPERTHYROIDISM

Observations were made for nine days following the administration of Ca^{45}.

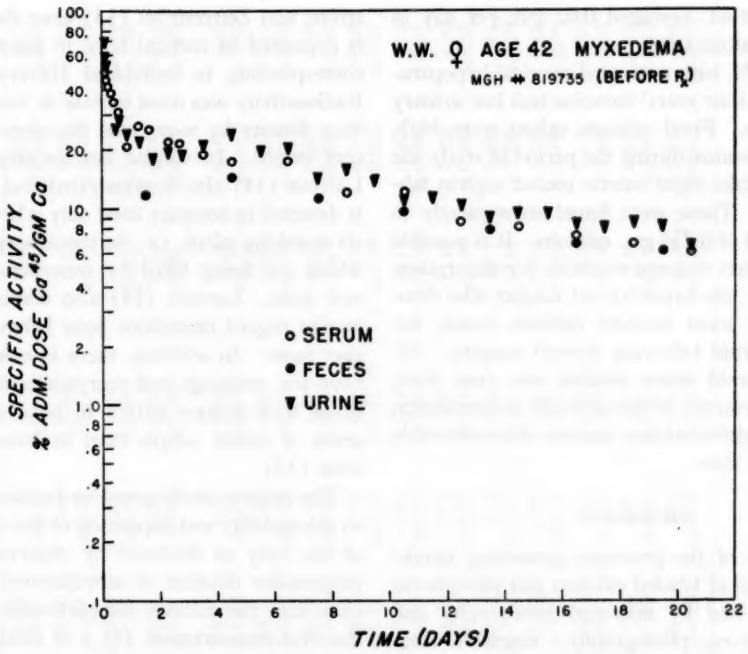

FIG. 4. SEMI-LOGARITHMIC PLOT OF SPECIFIC ACTIVITY VERSUS TIME FOR THE DATA FROM SUBJECT W. W.(1) WITH MYXEDEMA BEFORE TREATMENT

patient with long-standing hypoparathyroidism had the smallest, 5.1, 5.0, and 4.4 grams, respectively. After treatment of the myxedema, the calcium "pool" rose to 13 and 18 grams. The rapidity and extent to which labeled calcium enters body calcium spaces in hyperthyroid, euthyroid, and myxedematous subjects is illustrated in the calcium "pool" curves of Figure 6.

Balance data

Metabolic balance data for calcium, phosphorus, and nitrogen are given in Table II. Two of the four hyperthyroid patients (F. W. and J. B.) were in a distinctly more negative calcium and phosphorus balance than is normal for a low calcium intake. Both had high levels of calcium in the feces as well as in the urine. Both had elevated urinary excretion of nitrogen, but their intakes were high in nitrogen and calories. They remained almost in nitrogen equilibrium and maintained their body weight. The other two hyperthyroid subjects, both female, had normal urinary calcium values and were in only slightly negative calcium balance.[7] Alkaline phosphatase values in the serum were significantly elevated in patient F. W.(1), but there was no clinical evidence of liver disease, and there was no retention of bromsulphalein in the serum.

Urinary calcium excretion was low in both women with myxedema of long standing (B. M.(1) and W. W.(1)). The amount of calcium in the feces was normal. These subjects were in slightly negative calcium balance. Both were restudied five months after treatment with desiccated thyroid. Urinary calcium excretion had increased almost threefold, and total calcium excretion approximately doubled. The concentration of calcium and phosphorus in the serum was normal before and after treatment.

Subject L. S. went from negative calcium balance when she was hyperthyroid to positive balance five months after thyroidectomy, when signs of early myxedema were present. Urinary calcium, which had been 0.13 gm. per day when she

[7] Normal subjects are in slightly negative calcium balance on a low calcium intake (12).

was hyperthyroid, averaged 0.02 gm. per day in the myxedematous state.

Patient E. N. with untreated surgical hypoparathyroidism of four years' duration had low urinary calcium levels. Fecal calcium values were high, presumably because during the period of study she continued to take eight enteric coated aspirin tablets per day. These were found subsequently to contain a total of 0.254 gm. calcium. It is possible that this calcium was not available for absorption. Patient F. W., the hyperthyroid subject who demonstrated the most marked calcium losses, became parathyroid following thyroid surgery. Although euthyroid when studied one year later, he still had evidence of parathyroid underactivity. He was in positive calcium balance of considerable degree at this time.

DISCUSSION

The nature of the processes governing uptake by the skeleton of labeled calcium and phosphorus has been studied by microradioautography and comparative x-ray photography. Engfeldt, Engström, and Zetterström (13) have shown that P^{32} is deposited in cortical bone in discrete locations corresponding to individual Haversian systems. Radioactivity was most intense in those osteons of least density by x-ray and therefore of most recent origin. In similar studies employing Ca^{45}, Lacroix (14) also demonstrated that radioactivity is detected in compact bone only where osteogenesis is taking place, *i.e.*, in the absorption cavities which are being filled by concentric lamellae of new bone. Lacroix (14) also demonstrated that in this regard cancellous bone behaves like compact bone. In addition, there is evidence that *in vitro* ion exchange and recrystallization processes occur to a greater extent in bone crystals from areas of recent origin than in older established bone (15).

The present study provides further information on the mobility and deposition of the calcium stores of the body as disclosed by observations on the progressive dilution of administered labeled calcium into the calcium compartments. Mixing in the first compartment (Q_1) of dilution accounts

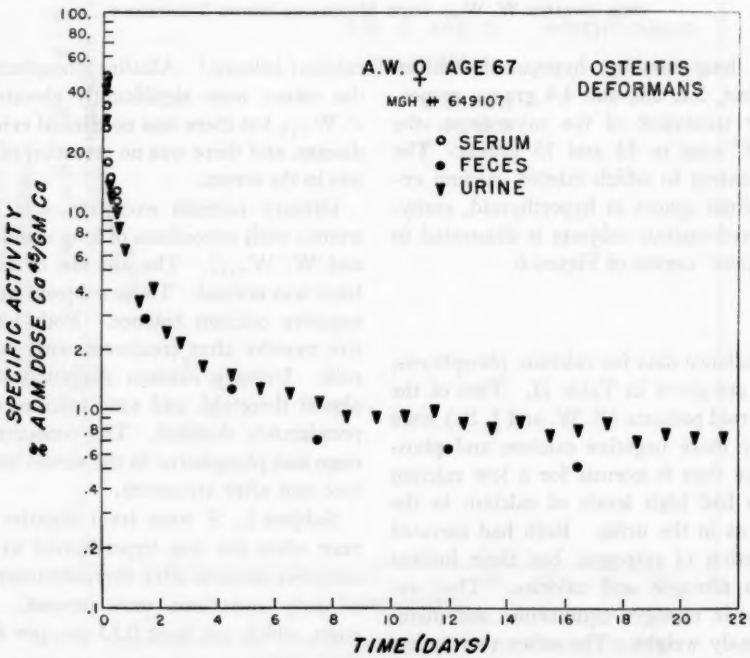

FIG. 5. SEMI-LOGARITHMIC PLOT OF SPECIFIC ACTIVITY VERSUS TIME FOR THE DATA FROM SUBJECT A. W. WITH EXTENSIVE PAGET'S DISEASE OF BONE

TABLE IV

Coefficients, rate constants, and compartments

Patient	A_1 (% adm. dose/Gm.)	K_1 (fraction/day)	Compartment 1 (Gm.)	Calculated E.C.F. Ca‡ (Gm.)	A_2 (% adm. dose/Gm.)	K_2 (fraction/day)	Compartment 2 (Gm.)	A_3 (% adm. dose/Gm.)	K_3 (fraction/day)	Compartment 3 (Gm.)
R. P. (A.S.H.D.)	82.73 ±16.27§	38.44 ±5.11	0.87 ±0.12	1.10	14.70 ±0.65	3.77 ±0.16	3.11 ±0.07	17.46 ±0.40	0.081 ±0.002	5.73 ±0.13
Wa. W. (Fr.A.)	37.33 ±5.44	28.26 ±4.46	1.30 ±0.10	0.88	23.80 ±1.93	1.94 ±0.21	2.53 ±0.13	15.80 ±0.72	0.164 ±0.010	6.33 ±0.29
F. W. (1) (Thyrtox.)	30.20 ±2.81	20.64 ±1.94	2.07 ±0.14	0.92	14.84 ±1.95	2.54 ±0.23	5.55 ±0.61	3.19 ±0.26	0.288 ±0.014	31.40 ±2.54
F. W. (2) (Hypoparathyr. after Rx.)	77.3 ±8.6	39.38 ±3.34	0.91 ±0.07	0.87	20.2 ±0.3	2.59 ±0.24	3.13 ±0.09	11.8 ±0.8	0.276 ±0.019	8.47 ±0.58
L. S. (1) (Thyrtox.)	66.55 ±20.50	36.60 ±9.31	0.92 ±0.18	0.81	28.21 ±1.88	3.20 ±0.05	2.40 ±0.12	13.38 ±0.97	0.279 ±0.015	7.47 ±0.54
L. S. (2) (Myx. after Rx.)				0.90	40.32 ±2.15	1.93 ±0.12	1.85 ±0.08	13.83 ±0.93	0.150 ±0.013	7.23 ±0.49
C. L. (Thyrtox.)	42.63 ±7.63	29.11 ±5.01	1.34 ±0.14	0.80	19.04 ±2.43	1.54 ±0.30	3.12 ±0.24	13.00 ±0.53	0.243 ±0.008	7.69 ±0.31
J. B. (Thyrtox.)	42.88 ±6.44	34.46 ±4.62	1.47 ±0.14	1.05	18.72 ±1.51	1.66 ±0.16	4.01 ±0.27	6.21 ±0.69	0.215 ±0.022	16.10 ±1.79
W. W. (1) (Myx.)	78.05 ±20.85	55.14 ±7.99	0.77 ±0.12	0.93	27.55 ±0.73	2.01 ±0.07	1.93 ±0.05	24.13 ±1.08	0.063 ±0.004	4.14 ±0.19
W. W. (2) (Norm. after Rx.)	67.41 ±21.13	39.05 ±9.39	0.86 ±0.16	0.77	24.86 ±3.95	1.75 ±0.37	2.07 ±0.18	23.43 ±1.27	0.150 ±0.006	4.27 ±0.23
B. M. (1) (Myx.)	74.27 ±12.85	32.31 ±5.18	0.77 ±0.08	0.97	27.63 ±3.92	3.16 ±0.32	1.81 ±0.14	27.56 ±1.45	0.072 ±0.005	3.63 ±0.19
B. M. (2) (Norm. after Rx.)	47.50 ±5.88	32.31 ±3.71	1.08 ±0.07	0.89	24.58 ±3.37	2.38 ±0.32	2.21 ±0.17	20.58 ±1.01	0.174 ±0.006	4.86 ±0.24
A. W.* (Paget's dis.)	80.85 ±28.22	31.66 ±8.14	0.99 ±0.28	0.66	15.76 ±1.91	1.54 ±0.36	4.95 ±0.49	3.32 ±0.61	0.436 ±0.034	22.57 ±1.93
O. H.† (Paget's dis.)	47.76 ±13.99	43.04 ±8.45	1.62 ±0.37	0.83	9.85 ±0.80	3.37 ±0.21	7.17 ±0.81	3.36 ±1.36	0.928 ±0.646	24.39 ±8.10
E. N. (Hypoparathyr.)	63.13 ±15.23	22.65 ±7.23	0.82 ±0.11	0.62	25.64 ±6.59	2.10 ±0.71	1.72 ±0.20	32.50 ±1.83	0.090 ±0.011	3.08 ±0.17

* A 4th exponent derived
$A_4 = 1.11 \pm 0.10$
$K_4 = 0.021 \pm 0.006$
Comp. 4 = 90.09 ± 7.95

† A 4th exponent derived
$A_4 = 0.74 \pm 0.04$
$K_4 = 0.11 \pm 0.01$
Comp. 4 = 135.14 ± 7.30

‡ E.C.F. Ca = extracellular fluid calcium content. This is calculated assuming that the serum volume = 0.05 × body weight; calcium in the serum = serum calcium concentration in grams per liter × serum volume; interstitial fluid calcium concentration = 0.7 × serum calcium concentration; interstitial fluid volume = 0.15 × body weight; interstitial fluid calcium = interstitial fluid calcium in grams per liter × interstitial fluid volume.

§ Standard deviation.

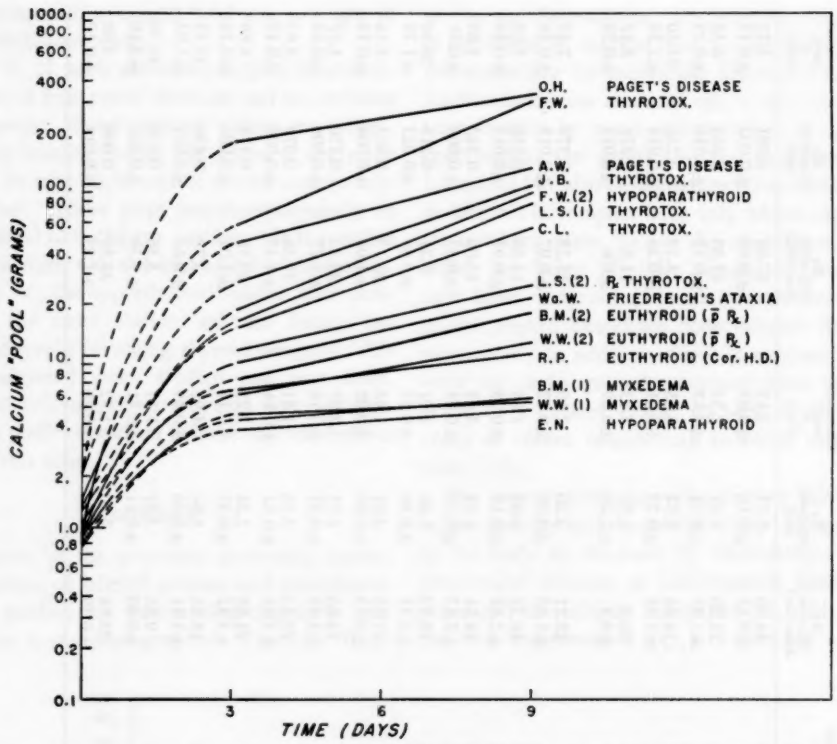

FIG. 6. CALCIUM "POOL" IN GRAMS $\left(\frac{\%\ \text{REMAINING IN BODY AT TIME t}}{\text{SPECIFIC ACTIVITY AT TIME t}}\right)$ IS PLOTTED SEMI-LOGARITHMICALLY VERSUS TIME

It is apparent that Ca^{45} in the hyperthyroid subjects exchanges with a larger quantity of calcium and more rapidly than normal.

for the initial rapid fall in serum specific activity of labeled calcium after intravenous administration. In view of the small size of this compartment and its high rate constant it probably represents extracellular fluid. In support of this assumption is the close agreement of the calculated values of calcium content of the extracellular fluid and those obtained from analysis of the curve of isotope dilution (Table IV). An insufficient number of blood samples were drawn in the first 30 minutes after injection of labeled calcium to distinguish between serum and interstitial fluid distribution.

The second and third compartments reflect reversible processes. Flow rates from the first to the second (k_1Q_1) and from the second to the third ($k_2\ [Q_1 + Q_2]$) are of too great a magnitude to be explained by an irreversible skeletal deposition of calcium salts, but are in keeping with ion exchange at the surface of bone crystals and equilibration of surface ions with those in the crystal interior, *i.e.*, recrystallization (15). Formation of new bone crystals incorporating the labeled calcium is consistent with flow ($k_3\ [Q_1 + Q_2 + Q_3]$) from the third compartment. Whatever their exact significance, the compartments and flow rates observed were sensitive to varying levels of thyroid gland function.

In all patients studied the exchange between the calcium in the extracellular fluid and that in bone was very rapid. Nevertheless, the concentration of stable calcium in the blood (and extracellular fluid) remained quite constant. The quantity of calcium exchanged was far greater than the *net* quantity of calcium transferred from the bone to the extracellular fluid. In those subjects in negative balance, the net calcium lost (excreted)

from the extracellular fluid was then rapidly replaced from the larger bone pools. This suggests part of the mechanism whereby serum levels can be maintained and controlled.

In all four hyperthyroid subjects the size of the calcium compartments and the rates of flow from these compartments were distinctly greater than in the euthyroid controls. This indicates increased osteogenesis. Calculation of the rate of calcium "accretion" from the data obtained in the present study by the method of Bauer, Carlsson, and Lindquist (16) supports this concept. Since these patients were in negative calcium balance on a low calcium intake, bone destruction as well as bone formation was proceeding at an increased rate. In myxedema, there was a slower rate of these processes. When myxedema was treated, the rate of fall of specific activity and the size of the compartments increased.

In hyperthyroidism, excessive destruction of bone may be the primary event. In addition, under the conditions of the present study, there may have been increased bone resorption in response to the low dietary intake of calcium. However, the weakened bones were *able* to respond with an increase in osteoblastic activity followed by deposition of mineral. This is what is pictured to occur in "osteitis fibrosa." Askanazy and Rutishauser (17) and, later, Follis (18) reported microscopic evidence of bone destruction in patients dying with hyperthyroidism. In Follis' series of 20 patients, osteitis fibrosa was found in the vertebral bodies of all, whereas osteoporosis was observed only in four subjects. These findings are thus not compatible with the concept that the primary bone disease of thyrotoxicosis is osteoporosis, defined as decreased bone formation from a failure of matrix formation, with bone destruction proceeding at a normal rate (19). The elevated concentration of alkaline phosphatase in the serum of patient F. W. when thyrotoxic also supports the concept of increased bone formation and is consistent with reported observations in other hyperthyroid patients (20) and the finding of increased bone phosphatase in animals given excessive doses of thyroid (21).

It is possible that nutritional or other factors may adversely affect bone formation in other patients with hyperthyroidism. All in the present study were adults. Those females with abnormal thyroid function were pre-menopausal. Age may have been a factor in that the younger individuals studied here conceivably had a greater capacity to respond with an increase in osteogenesis. The two hyperthyroid subjects, F. W. and J. B., who were in the most negative calcium balance, were nearly in nitrogen equilibrium. This fact is contrary to the view that the hypercalcuria of hyperthyroidism is directly related to nitrogen losses (22).

The turnover of calcium in the bones may be related to blood flow. For this reason, two patients with Paget's disease of bone were studied. Often in osteitis deformans there is an increase in cardiac output and bone blood flow (23). The increase in vascularity could involve a greater exposure of bone crystals to Ca^{45} in circulating fluids and thereby increase the exchangeable surface. Both patients demonstrated an increased skeletal turnover of calcium. Both had extensive disease and elevation of the serum concentration of alkaline phosphatase. Increase in the vascularity of the skeleton in hyperthyroidism could influence skeletal uptake in the same direction, whereas decreased vascularity could have the opposite effect in myxedema. Blood flow in other organs is known to be decreased in myxedema (24).

Relief of the hypothyroid state was followed by a decreased turnover of skeletal calcium. Patient F. W., who demonstrated marked calcium losses and rapid turnover of skeletal calcium when hyperthyroid, still showed a rapid fall in specific activity one year after thyroidectomy, when euthyroid but hypoparathyroid. At the time of the second study, the size of the calcium compartments was much smaller and the calcium "pool" at nine days considerably decreased compared to the initial study. Nevertheless, the values were higher than expected by comparison to other subjects studied. It is possible that this patient had not remineralized his bones completely at the time of the second study.

Similarly, the hyperthyroid subject, L. S., restudied when myxedematous four months after thyroidectomy, showed a decrease by a factor of two in rates of flow of calcium in and out of the osseous compartments, but the values were then about the same as the euthyroid controls. At the same time, urinary and fecal calcium excretion were reduced by a factor of four. This implies

that after withdrawal of the thyroid hormone, a decrease in the rate of bone destruction occurs sooner than a decrease in osteogenesis.

The effects of thyroid hormone are probably not mediated by the parathyroids. Aub, Albright, Bauer, and Rossmeisl (4) demonstrated that thyroid increases the serum and urinary calcium in patients with idiopathic and surgical hypoparathyroidism. Cope and Donaldson (5) reported a patient who developed hypocalcemia and tetany following an inadequate subtotal thyroidectomy for hyperthyroidism. When hyperthyroidism recurred, tetany was relieved and the concentration of calcium in the serum rose. Hypocalcemia and tetany again appeared after treatment with potassium iodide.

Increased fecal calcium in two patients with hyperthyroidism and the very low fecal calcium in one of the myxedematous subjects (L. S.$_{(2)}$) are in keeping with the earlier reports of Aub, Bauer, Heath, and Ropes (1) and are contrary to the concept proposed by Robertson (25) that the primary effect of the thyroid hormone on calcium metabolism is on the kidney alone. The finding of hypercalcuria, often extreme in some patients with hyperthyroidism, in the presence of normal serum calcium suggests either an increase in filtered load or decreased tubular reabsorption.

SUMMARY AND CONCLUSIONS

1. Radioactive calcium (Ca^{45}) was given intravenously to patients with hyperthyroidism and myxedema. Euthyroid subjects and patients with Paget's disease of bone, and others with hypoparathyroidism were similarly studied. Several patients were studied before and after appropriate treatment. Total quantities of the isotope excreted and specific activities of the serum, urine, and feces were determined frequently for 9 to 22 days. In addition, stable calcium, phosphorus, and nitrogen balances were performed on each subject.

2. In four subjects with hyperthyroidism, specific activity of serum and urine declined more rapidly and to lower levels than in euthyroid controls. In contrast, two myxedematous patients demonstrated the least fall in specific activity. After five months of treatment of the myxedema specific activity curves were found to decline at the same rate as in the control patients.

3. Observation of all the specific activity curves suggests that they can be analyzed into a series of decreasing exponential functions. It is proposed that each function represents a discrete calcium compartment within the body.

4. Changing thyroid function modifies the size of the calcium compartments and the rate of flow to and from these compartments. Compartment sizes and flow rates are greatest in hyperthyroidism and Paget's disease of bone and least in myxedema.

5. Two of four hyperthyroid subjects were in nitrogen equilibrium, despite which they showed marked losses of calcium in the urine and feces.

6. The findings indicate that bone formation as well as destruction are proceeding at increased rates in thyrotoxicosis.

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THE RENAL TUBULAR RESPONSE TO AMINO ACID LOADING[1]

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(Submitted for publication February 1, 1956; accepted April 24, 1956)

In previous studies from this laboratory (1) it was shown that renal tubular reabsorption of filtered amino acids was virtually complete in the fasting state. However, when a solution of amino acids was administered intravenously and the load filtered by the kidney thus increased, both the amounts of amino acids reabsorbed and those excreted were increased. In quantitative terms the more noteworthy event was the increase in amount reabsorbed. Considerable selectivity was exhibited in the reabsorption of individual amino acids and evidence was obtained which was interpreted as indicating a "competition" for reabsorption between cystine and one or more other amino acids.

In the present studies the solution of amino acids used for loading was administered at a faster rate than in the previously reported experiments and it was supplemented with additional glycine to test the effects of a further increase in the filtered load of amino acids. In separate studies glycine was administered alone and, in terms of amounts reabsorbed and excreted, the results were compared with those obtained when glycine was given as part of the amino acid mixture and comparable filtered loads were achieved.

METHODS

Three normal subjects—DNW (age 21, ht. 184.2 cm., wt. 85.5 kg.), WRR (age 28, ht. 175.3 cm., wt. 95.0 kg.), and WS (age 29, ht. 168.9 cm., wt. 60.5 kg.)—were given Merck's 10 per cent solution of free amino acids. The experimental protocol is outlined in Table I-A, the rate of infusion being calculated on a basis of its glycine content. The glomerular filtration rate (GFR) was measured by means of inulin clearance and the manner in which the blood and urine specimens were collected was the same as that used in previous studies (1). The designation "high load" is an arbitrary one, meaning not only that the infusion was given at a faster rate but in addition, in the case of the first three experiments, that the amino acid mixture was supplemented with additional glycine.

Three normal subjects—WRR, BVS (age 27, ht. 167.6 cm., wt. 81.7 kg.), and DMS (age 23, ht. 167.6 cm., wt. 95.5 kg.)—received glycine alone, as outlined in Table I-B.

The methods employed for the microbiological assays of the individual amino acids and the exact composition of the amino acid solution used, as determined in this laboratory (lot B-27), have been reported (1, 2). The statistical analysis of the data in which each individual's control period measurements were available was made by means of the 't' test for paired data. A p value of 0.05 was accepted as the criterion of significance (3).

RESULTS

Over-all response of the kidney to the infusion of an amino acid mixture

In Figure 1 the sum of the filtered load of all of the individual amino acids has been plotted against the total amount reabsorbed. The first two points represent mean and grand mean values, respectively, obtained in six experiments. The last point is a grand mean value obtained from the individual high load periods run in three experiments. The same general pattern of renal response noted earlier by us with lower total filtered loads and by other investigators (4–8) is evident; *i.e.*, as the filtered load is progressively increased both the amounts reabsorbed and those excreted are increased. The increase in the amount reabsorbed, however, is not equally proportionate to the increase in that filtered, and as a result the per cent reabsorbed decreases.

Apparent differences in reabsorption patterns among groups of amino acids

The mean and grand mean amounts of all the amino acids, except cystine, filtered and reab-

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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TABLE I

Experimental protocol

Subject	Control 1 2 3	Load* 4 5 6	High Load* 7 8
		μM glycine/Kg./min.	
A. Infusion of amino acid solution#			
IBV		15.8	39.3
WHR		13.3	33.4
WS+		19.9	43.2
B. Infusion of glycine alone			
WHR		13.2.	
BYS		17.1	
DMS		24.4	49.7

* A 100-ml. priming dose of the particular solution being studied was administered rapidly when the infusion was started and 15 min. were allowed for equilibration. In subject DMS, a 60-ml. priming dose was used.

The supplies of amino acids solution were generously provided by Merck & Co., Inc.

+ In subject WS the protocol was modified in that 2 load and 3 high load periods were run.

sorbed by the three subjects in the fasting, load, and high load periods, are graphically presented in Figure 2. In the case of the first ten amino acids (reading from the left of the figure), it will be noted that the amounts reabsorbed continued to increase as the filtered load was progressively elevated. This is similar to the overall response made by the kidney to an infusion of an amino acid solution and is what would be predicted for the behavior of the individual amino acids. However, when the load and high load periods are compared in the case of glycine, serine, threonine, and histidine, it will be noted that despite moderate to striking increases in the filtered load the absolute amounts reabsorbed were not significantly different in the high load than in the load periods. In fact, the amount of histidine reabsorbed in the high load period was significantly less than that reabsorbed in the load period.[5]

In Table II the glomerular filtration rate values for all of the subjects and the plasma concentration and excretion values obtained in the case of histidine are listed to provide some information as to the stability of the experimental system as well as to show the increase in histidine excretion. The decrease which occurred in histidine reabsorption can be calculated from these data.

When the amounts of glycine and histidine reabsorbed in the individual high load periods were analyzed, a tendency to decreased reabsorption in successive periods was observed. This same trend was reflected in the total amount of all amino acids reabsorbed in each period (Figure 3) and although in this instance the changes are not statistically significant, the observation is mentioned because of its important implications.

Effect of amino acid loading on the clearance of cystine

In a previous study (1) it was observed that the clearance of cystine increased during the infusion of an amino acid mixture in the absence of any increase in filtered load of cystine. The infusion mixture is virtually free of cystine (lot B-27 contains 0.09 μM per ml.). In Table III there are presented the absolute amounts of cystine filtered and reabsorbed, as well as the clearance of cystine during each of the three periods in the present study. Again it will be noted that the clearance increased during the load period and a further increase was observed in the high load periods. This occurred in the absence of any statistically significant increase in the filtered load of cystine. It is noteworthy that the mean clear-

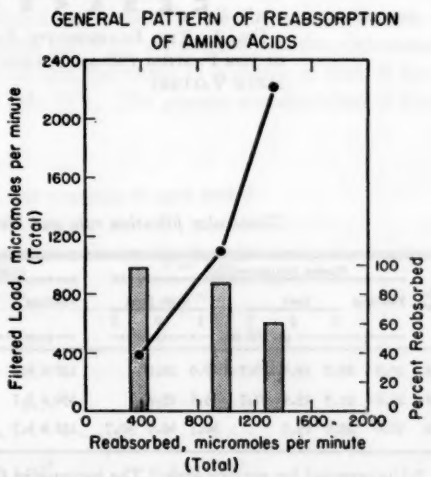

FIG. 1. THE GENERAL PATTERN OF REABSORPTION OF AMINO ACIDS

[5] It is not possible, on the basis of the results obtained in these experiments with respect to their reabsorptive mechanism, to state in which group of amino acids tyrosine and glutamine should be included.

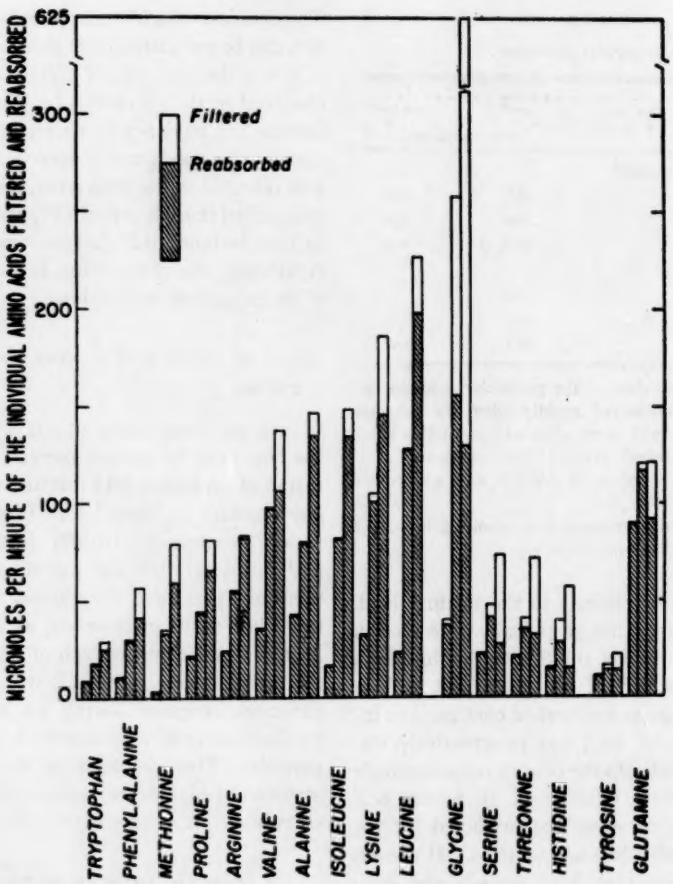

FIG. 2. THE REABSORPTIVE PATTERN OF THE INDIVIDUAL AMINO ACIDS IN THE FASTING (MEAN VALUES), LOAD, AND HIGH LOAD PERIODS (GRAND MEAN VALUES)

TABLE II

Glomerular filtration rate and histidine values in each subject for all periods

Subject	Plasma Concentration							Glomerular Filtration Rate*							Excretion						
	Fasting	Load			High Load			Fasting**	Load			High Load			Fasting	Load			High Load		
		1	2	3	1	2	3		1	2	3	1	2	3		1	2	3	1	2	3
	μM/100 ml.							ml./min.							μM/min.						
MRR	10.3	20.7	19.4	18.7	29.0	31.0		138 ± 4.4	162	163	167	171	164		1.12	13.0	11.9	11.7	36.8	41.9	
DMV	11.6	21.3	23.9	27.7	33.6	25.2		150 ± 8.7	168	175	168	184	183		0.54	8.3	5.8	6.3	29.9	35.8	
WS	10.6	29.0	29.0		40.7	40.0	40.7	132 ± 4.7	166	154		169	164	166	1.0	16.4	15.6		46.5	52.3	61.3

* Uncorrected for surface area. The increase in GFR sometimes noted with these infusions has been commented upon (1).

** Mean value and standard deviations derived from control periods.

ance (32.6 ml. per min.) achieved during the high load periods approaches 24-hour clearance values observed by us in some patients with cystinuria.

Comparison of the excretion of glycine when infused alone and when incorporated into an amino acid mixture

In Figure 4 the amounts of glycine excreted when it was administered alone are compared with the excretion when it was infused as part of an amino acid mixture and comparable filtered loads achieved. The four columns on the left of the figure represent mean values obtained in four experiments, two wherein glycine was infused alone (subjects WRR and BVS), and two wherein it was part of the amino acid mixture (subjects WRR and WB). In the four columns on the right, the results obtained in a single experiment with glycine alone are compared with those obtained in three experiments wherein the amino acid mixture was infused and comparable filtered loads of glycine achieved. The toxicity of this high dose of pure glycine prohibited further study on other subjects and will be commented on later. Significantly more glycine was excreted when it was infused as part of a mixture and high filtered loads obtained ($p = 0.01$) and although the results obtained at lower filtered loads are not statistically significant, the tubular response appears to be the same.

During the infusion of the glycine solution the clearances of other amino acids were also measured. In subject BVS the clearances of 17 amino acids were measured. In the other two

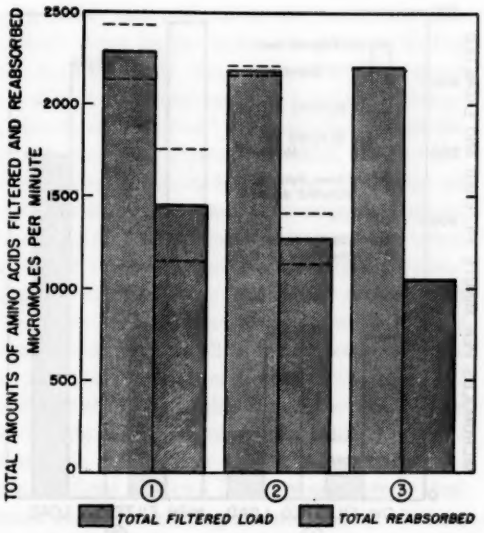

FIG. 3. THE TOTAL AMOUNT OF ALL THE AMINO ACIDS FILTERED AND REABSORBED IN EACH OF THE THREE HIGH LOAD PERIODS

The first and second periods are graphed as mean values with the standard deviations (dotted lines) obtained in the three subjects. The third represents the values obtained in a single subject (WS).

experiments (WRR and DMS) only amino acids representative of the types with high (histidine, serine, and threonine) and low (leucine, lysine, and valine) clearance values were measured. The infusion of glycine in all three experiments was accompanied by an increase in the clearances of serine and threonine in addition to that of glycine (Table IV). The plasma concentration of glycine

TABLE III

Cystine—Amounts filtered, reabsorbed, and clearance in each period (mean values)

Subject	Fasting		Load		High Load	
	Filtered	Reabsorbed	Filtered	Reabsorbed	Filtered	Reabsorbed
	μM/min.		μM/min.		μM/min.	
DMW	16.3	16.1	17.1	16.1	15.4	14.0
WRR	13.8	13.6	16.2	15.5	16.6	12.0
WS	12.1	11.9	14.9	14.4	16.4	12.9
Mean Clearance ml./min.	1.92 ± 0.3*		5.97 ± 1.27*		32.6 ± 17.3*	

* Standard deviation.

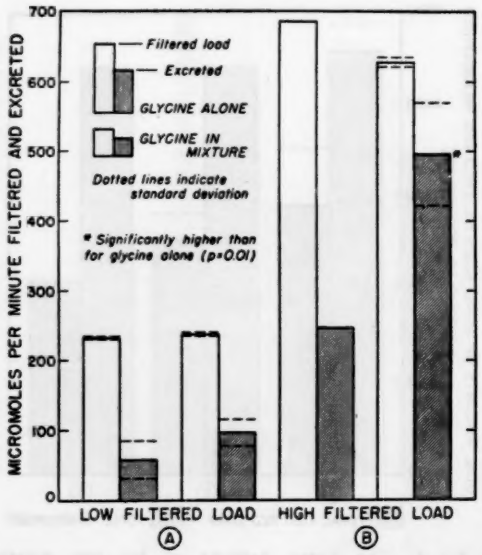

FIG. 4. COMPARISON OF AMOUNTS EXCRETED WHEN GLYCINE WAS INFUSED ALONE AND AS PART OF AN AMINO ACID MIXTURE

rose in all of the subjects; but in DMS, who received the largest amounts, there was, in addition, a threefold elevation in plasma-serine concentration (15.2 to 41.9, 47.6, and 45.7 μM per 100 ml.) and a twofold elevation in plasma-threonine concentration (14.3 to 28.6, 29.4, and 29.4 μM per 100 ml.). No changes were observed in plasma concentration or clearance in any of the other amino acids during the glycine infusion.

DISCUSSION

General features of the amino acid reabsorptive mechanism

As the filtered load of a single amino acid or of a mixture of amino acids is increased, both the amounts reabsorbed and excreted increase. In this respect the reabsorption of amino acids resembles substances such as urate in man (9) and xylose in the dog (10). Reabsorption itself most certainly involves "active transport" and as such requires energizing reactions. However, the specific reactions concerned and the relation they bear to the rate of transport have not yet been described. The present studies have shown that striking differences in rates of reabsorption exist among the individual amino acids, a fact which may be explained by assuming that they are handled by independent mechanisms or by the same mechanism at different rates. It is predicted that in the absence of toxic or antimetabolite phenomena the maximal rate of reabsorption for a mixture of amino acids would vary with the qualitative as well as the quantitative characteristics of the particular mixture being infused, "mixture Tm," while the conventional or true tubular Tm would be attained only if the mixture were constituted in such an ideal manner that both the affinity for and the capacity of the transporting system were maximally utilized.

In the instances wherein Tm values have been demonstrated for individual amino acids in the dog (11, 12), the saturating value has been attained in a gradual manner and undoubtedly a similar gradual approach would be demonstrated for a mixture of amino acids in human subjects. Pitts (11) has explained this reabsorptive pattern within the framework of Shannon's heuristic hypothesis on the law of mass action as applied to the cellular transporting reaction (13). According to Pitts, the limiting reabsorptive maxima are attained gradually because the velocity of decomposition of an amino acid complex (AB) within the tubular cell is relatively rapid in relation to the attainment of equilibrium in the reaction by which it is formed (A, reabsorbed material and B, some stable cellular component, constant in total amount), $A + B \rightleftarrows AB \rightleftarrows B + A$. Decomposition of the complex AB delivers the reabsorbed material to the peritubular blood.

However, an alternative explanation for the

TABLE IV

Changes in clearance of other amino acids noted during the infusion of glycine alone

Amino Acid	Clearance	
	Fasting Mean	Infusion Grand Mean
	ml./min.	
Glycine	8.8 ± 4.5	34.3 ± 7.5*
Serine	1.26 ± 1.1	12.6 ± 3.5*
Threonine	0.5 ± 0.7	3.1 ± 1.1+

* $p = 0.01$
+ $p = 0.05$

gradual approach to Tm is that among the nephron population there exists a considerable variation in functional capacity to reabsorb amino acids. As a result, varying percentages of the population would become saturated at different plasma levels and the level at which any given nephron or group of nephrons is to become saturated would be a function of the glomerular-tubular balance and/or the distribution of enzymatic activity within the tubular cells. The normal situation which obtains with amino acids might conceivably be analogous to that existing in certain cases of renal glycosuria wherein the maximal reabsorptive capacity for glucose is normal despite the fact that glucose is excreted at relatively low plasma levels (14, 15). The existence of two distinct groups of nephrons appears to be the most satisfactory way of explaining this normal Tm glucose (15).

In human subjects it is impossible to collect the type of mean data necessary for a definitive evaluation of either of these hypotheses. On the basis of certain indirect evidence, namely, the competition existing among individual amino acids for reabsorption in the normal subject and the fasting and load excretion values for individual amino acids in patients with various types of aminoaciduria, we believe that variation in functional capacity among the nephron population is neither the sole nor the most important determinant of reabsorptive pattern.

Specific features of the reabsorptive mechanisms

When the results with the individual amino acids were expressed as grand mean values, certain distinct differences in reabsorptive pattern were noted. Tryptophan, valine, proline, phenylalanine, methionine, lysine, leucine, isoleucine, arginine, and alanine continued to be reabsorbed in greater amounts as their filtered loads increased. The reabsorption of glycine, serine, and threonine, on the other hand, was not significantly different in the load and high load periods despite moderate to striking increases in their respective filtered loads. Moreover, in the case of histidine, a significantly smaller amount was reabsorbed in the high load periods. The reabsorptive capacity for the first group of amino acids obviously was not saturated despite the increases in load, corroborating the findings of earlier studies made at lower plasma levels and reaffirming the prediction that whenever these amino acids are present in the urine in abnormally large amounts, it is more likely due to an impairment in tubular reabsorption than to an "overflow" phenomenon. When the data obtained with glycine, histidine, serine, and threonine are interpreted from the standpoint of the grand mean values, the results suggest that under these experimental conditions some one other or group of other amino acids was successfully competing for or interfering with their reabsorptive mechanisms. The results obtained when glycine was infused alone, as well as the evidence accumulated in a number of other studies (5, 11, 16, 17), all indicate that competition for reabsorption takes place among the individual amino acids.

Although competition undoubtedly exists, evidence has been acquired in the present study that another event is occurring simultaneously. When the amounts of glycine and histidine reabsorbed in the individual high load periods were reviewed, a tendency to reabsorb less in successive periods was observed in each of the three subjects. The actual values were not significant in the case of glycine and of borderline significance ($p = 0.06$) for histidine. This same trend was observed in the total of all the amino acids reabsorbed in each individual high load period. In Figure 3 is presented the total for all the amino acids filtered and reabsorbed as mean values for each of the periods and the trend to reabsorb less with time will be noted. The apparent decrease in total amount reabsorbed plus the fact that the decrease was largely but not completely accounted for by the decrease in glycine and histidine reabsorption suggests that a decrease with time, in reabsorptive capacities for glycine, histidine, and perhaps other amino acids had taken place independently of competitive shifts in reabsorption pattern. This decrease may represent a "fatiguing" of the reabsorptive mechanisms due to an excessive concentration of reaction products, an antimetabolite, or perhaps a toxic phenomenon. The untoward reactions (malaise, nausea, and vomiting) associated with these infusions were sufficiently severe as to deem it imprudent to perform further experiments wherein higher plasma levels would be sustained for longer periods. Animal experiments specifically designed to determine whether any decrease

in reabsorptive capacity occurs with time are currently in progress.

The tubular response to the administration of glycine

Glycine was administered as a 2.5 and as a 5.0 per cent solution to two and to one normal subjects, respectively. Greater amounts were reabsorbed when it was thus infused than when it was given as part of an amino acid mixture and comparable filtered loads achieved. In all three subjects there was a significant rise in the clearances of serine and threonine as well as glycine. Increases in the excretion of amino acids other than the one being infused have been observed by others. Beyer and his co-workers (16) originally demonstrated the relationships existing in the reabsorption of leucine and isoleucine as well as arginine, histidine, and lysine. In these same studies no significant competition for reabsorption was demonstrated between glycine and isoleucine, leucine and arginine. Handler, Kamin, and Harris (18), however, observed a definite increase in arginine and a marked increase in methionine, isoleucine, tryptophan, histidine, and threonine excretion when glycine was infused into dogs at a rapid rate (3.0 mg. glycine nitrogen N per kg. per min.). Glycine was administered at much slower rates in the present studies (0.26 to 1.03 mg. N per kg. per min.) and no significant increase in histidine, leucine, lysine, valine, alanine, arginine, cystine, isoleucine, methionine, phenylalanine, proline, tryptophan, or tyrosine excretion was observed. Kamin and Handler's studies indicated that histidine and threonine reabsorption could be inhibited most easily by the infusion of other amino acids (17).

In DMS, the subject who received the 5.0 per cent solution of glycine, there was a threefold rise in plasma serine and a twofold elevation in plasma threonine concentration. The rise in plasma serine was not surprising in view of the fact that conversion of glycine to serine is a metabolic route for the disposal of glycine, but the rise in plasma concentration of the essential amino acid, threonine, was unexpected and remains unexplained. No decrease in the plasma concentration of other amino acids, such as Hier (19) observed following the oral ingestion of leucine and isoleucine, was observed in this study.

The implications of the results obtained with glycine must be considered in the knowledge that this amino acid is in certain respects unique. It is the only naturally occurring amino acid which lacks an asymmetric carbon atom and therefore is incapable of isomerization; furthermore, its oxidative deamination in liver and kidney is accomplished by a specific enzyme, glycine oxidase, which catalyzes its conversion to glyoxylic acid. For these reasons the findings reported with respect to glycine cannot necessarily be extended to the other amino acids.

The toxicity associated with glycine infusions

The infusion of 2.5 per cent glycine was accompanied by a feeling of malaise and nausea in both subjects but the reaction was considered mild. The subject (DMS) who received the 5.0 per cent solution experienced a severe reaction. Approximately 45 minutes after the infusion was begun at 0.44 mg. N per kg. per min., he complained of nausea; 75 to 80 min. after the infusion was started and shortly (15 to 20 min.) after the infusion rate had been increased to 1.03 mg. N per kg. per min., he complained of malaise and began to vomit. The patient appeared quite ill by the time the infusion was discontinued at 104 min. The mean infusion rate was 0.67 mg. N per kg. per min. (the priming doses of glycine are included in calculating the above three infusion rates). The reaction was complicated by what appeared to be a pyrogenic reaction, temperature 100.8°F., shaking chill, and headache. The malaise, marked weakness, intense nausea, and vomiting persisted for about 9 hours post-infusion. A decrease in GFR occurred during the last two collection periods and the glycine data collected during that period have not been included in any of the results discussed above.

Pitts (11) observed a decrease in glomerular filtration rate and renal plasma flow, vomiting, dilatation and fixation of the pupils, weakness, and incoordination in dogs given 2 to 10 per cent solutions of glycine. These reactions were reversible within a few hours. Simultaneous elevation of plasma glucose and glycine produced near fatal reactions with sharp falls in GFR, coma, and

decerebrate rigidity. In the experiments of Handler, Kamin, and Harris (18), 8 of 12 dogs expired from respiratory failure when given glycine or glycine plus glucose at a rate of about 1.0 mg. N per kg. per min. The time at which death occurred was inversely proportional to the rate of administration so that toxicity best correlated with total dose rather than the duration of infusion. The fatal dose was quite variable, 8 of the dogs succumbing after receiving 360 to 1301 mg. N per kg. and 4 surviving after receiving doses ranging from 703 to 1970 mg. N per kg.

The rate at which the infusion is administered may be more important than Handler, Kamin, and Harris' results would imply, for in subject DMS toxicity became much more apparent and severe shortly after the infusion rate was increased to 1.03 mg. N per kg. per min. The total dose he received was only 69.7 mg. N per kg. If glycine is given at such a rapid rate that the rate of deamination exceeds the rate of removal of ammonia (urea formation), deaths from ammonia intoxication could result. Evidence in support of this explanation has been acquired by the authors in three separate experiments performed on dogs wherein blood ammonia concentrations have been serially determined during the infusion of glycine in lethal quantities. At the time of death the blood ammonia was found to be markedly elevated. These studies are being continued in an attempt to define more completely the mechanisms concerned.

SUMMARY

An amino acid mixture, supplemented with additional glycine, was administered at a faster rate than employed in earlier studies in order to determine the effect of the additional increase in the total filtered load of amino acids.

As the total filtered load of amino acids was raised to 2200 μM per minute, greater quantities were both reabsorbed and excreted.

The amounts of alanine, arginine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, tryptophan, and valine reabsorbed continued to increase as their filtered loads were elevated.

On the other hand the amounts of glycine, serine, and threonine reabsorbed were no greater in the high load than in the load periods and in the case of histidine significantly less reabsorption occurred. A suggested explanation for this latter finding is the simultaneous operation of two factors, competition among individual amino acids for reabsorption and a "fatiguing" of specific transport systems.

Although during the infusion of the amino acid mixture the filtered load of cystine was not changed, its clearance increased to a maximum of 32.6 ml. per min., a value aproaching that which we have observed in some patients with cystinuria.

Glycine, when infused alone, was more completely reabsorbed than when incorporated into an amino acid mixture and comparable filtered loads achieved. The infusion of glycine was accompanied by an increase in the excretion of serine and threonine and in one instance by an elevation in plasma concentration of serine and threonine.

The infusion of glycine proved to be toxic and separate data were collected indicating that this may have been due to ammonia intoxication.

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THE EXPERIMENTAL PRODUCTION OF ASCITES IN THE DOG WITH DIABETES INSIPIDUS[1]

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(Submitted for publication November 22, 1955; accepted April 3, 1956)

The role of the antidiuretic hormone of the neurohypophysis in the formation of edema remains to be clarified. Many investigators (1–4) have presented evidence which suggests that endogenous antidiuretic hormone may be responsible for the production and maintenance of various edematous states, especially the ascites of liver disease. The urine of patients with toxemia of pregnancy, nephritic edema, Cushing's syndrome, congestive heart failure, nephrosis, and cirrhosis of the liver with ascites, frequently has been found to contain increased amounts of an antidiuretic substance possibly secreted by the neurohypophysis.

Ralli and her group were perhaps the first of many workers to postulate a causal relationship between an increased liberation or persistence of antidiuretic hormone and the formation of ascites in hepatic cirrhosis (5, 6). As a result of studies on the control of ascites in this disease, Eisenmenger, Blondheim, Bongiovanni, and Kunkel (7, 8) attributed the beneficial effect of a dietary restriction of sodium to a decreased liberation of antidiuretic hormone. Shorr and his associates studied 23 patients with edema and found that the concentration of VDM (ferritin) in the blood was uniformly increased (9). These workers therefore postulated that the oliguria of heart failure, cirrhosis and nephrosis is the result of hypersecretion of antidiuretic hormone induced by VDM. In reviewing the problem of edema formation, Peters (10) pointed out that in the nephrotic syndrome as well as in cirrhosis and heart failure the serum sodium tends to be low. He believed that this hyponatremia is the result of a tendency to retain water in excess of salt and could be produced by an increased level of circulating antidiuretic hormone. Until recently, evidence that the antidiuretic hormone is inactivated by the normal liver had given implied support to the concept of abnormal persistence rather than hypersecretion of the hormone in cirrhosis (11). However, observation on patients (12) as well as *in vitro* studies (13) have failed to demonstrate inadequate hormone inactivation in this disease.

A major objection to the studies in which an increase in an antidiuretic factor in the urine was detected, is the failure to recognize the lack of specificity of the assay methods employed. van Dyke, Ames, and Plough (14) using a more specific intravenous assay in dogs to investigate the presence of antidiuretic hormone in the urine of patients with cirrhosis could not demonstrate a significant difference between a group with ascites and one without. In addition, in the various edematous states characterized by retention of salt and water, it is not clear whether the salt retention or the water retention occurs first.

The present study was designed to determine the role of the antidiuretic hormone in the formation of experimental ascites. In three dogs with known diabetes insipidus induced by interrupting the hypothalamico-hypophyseal tract in the hypothalamus, and in two control dogs, ascites was produced by constriction of the inferior vena cava above the hepatic vein by a modification of the method of McKee, Schilling, Tishkoff, and Hyatt (15). No significant difference was observed between normal dogs and dogs with diabetes insipidus in the capacity to form ascites after caval constriction. This report summarizes the results of electrolyte and water balance studies and of assays for anti-

[1] This work was supported in part by research grants from the U. S. Public Health Service, National Heart Institute (USPHS H-1275 and H-1788).

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[4] Research Assistant, supported by a research grant (H-1788) from the U. S. Public Health Service, National Heart Institute.

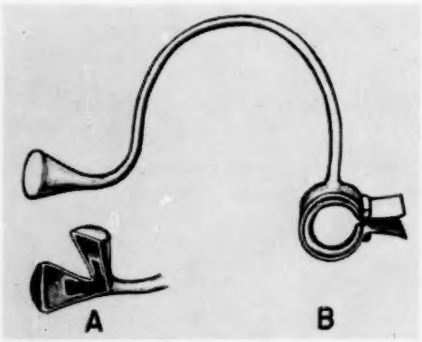

FIG. 1. INFLATABLE CUFF FOR CONSTRICTION OF THE VENA CAVA. A. SECTION OF BLIND END IMPLANTED SUBCUTANEOUSLY. B. CUFF APPLIED AROUND VESSEL

diuretic hormone in the hypothalamus and posterior lobe of the pituitary.

METHODS

The experiments were carried out with trained unanesthetized mongrel dogs, housed in metabolism cages and fed a standard diet of fixed electrolyte content. Some dogs were fed "Kasco" dog food in known amounts, constant for a given dog. Others were fed ground beef with added sodium chloride. Each new batch of food was analyzed for its content of sodium, potassium, and chloride. The electrolyte content of any uneaten residue was determined and subtracted from the daily intake. Distilled water was given *ad libitum*, and the daily consumption was measured. Urine collections were made every twenty-four hours. Stool specimens were not analyzed. None of the dogs had diarrhea at any time. The dogs were weighed daily in the fasting state. Blood was taken from the jugular vein with a minimum of stasis and delivered under oil. The serum was analyzed for sodium, potassium, and chloride; total protein and urea nitrogen were determined by methods described previously (16, 17). The urine was analyzed daily for sodium, potassium, and chloride. The specific gravity of each twenty-four-hour sample was determined with a standard hydrometer.

Constriction of the vena cava was carried out after a control balance study of at least ten days, and the dogs were returned directly from the operating room to the metabolism cage. The constriction of the vena cava was accomplished by a modification of the method described by McKee, Schilling, Tishkoff, and Hyatt (15) using a pneumatic rubber cuff devised by one of the authors (J. J.) and shown in Figure 1. By injecting or withdrawing air from the rubber cuff through a blind end

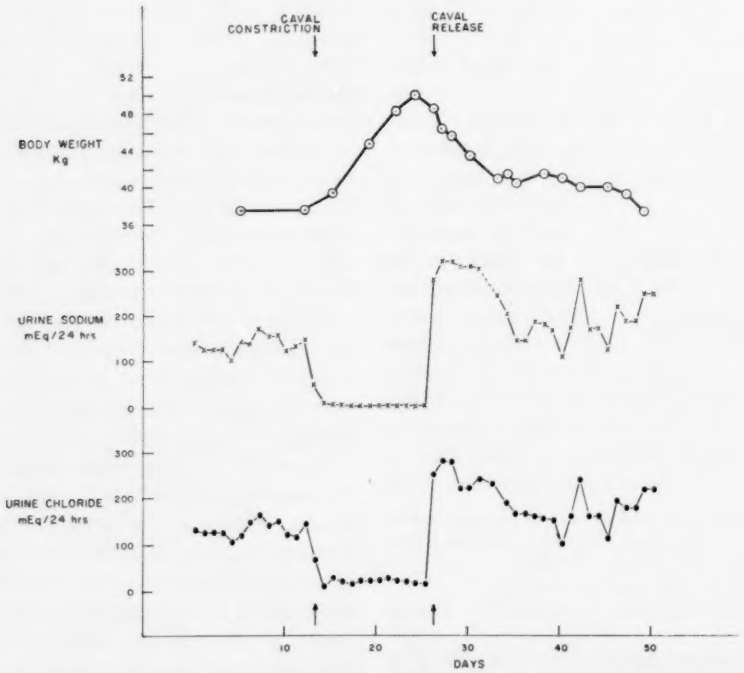

FIG. 2. PRODUCTION OF REVERSIBLE EDEMATOUS STATE IN A DOG ON CONSTANT NaCl INTAKE

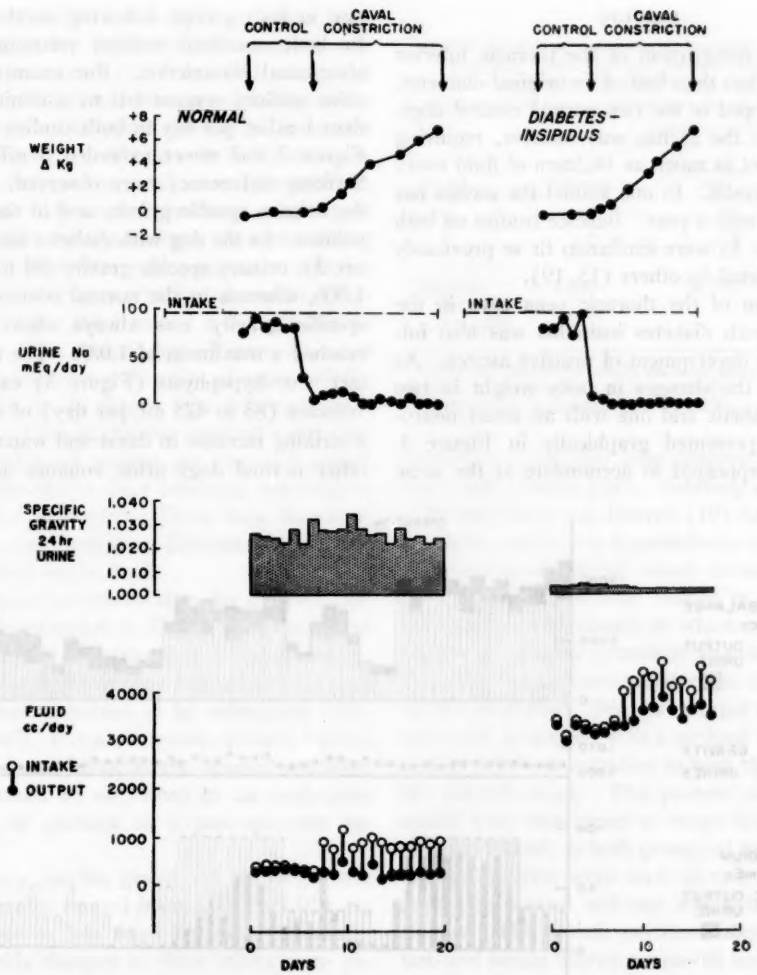

FIG. 3. FORMATION OF ASCITES IN A NORMAL DOG AND A DOG WITH DIABETES INSIPIDUS

implanted subcutaneously it is possible to produce a state of reversible, chronic ascites in the dog with diabetes insipidus (Figure 2) as well as in the control animals. Inflation and deflation of the previously inserted cuff through a No. 25 needle is accomplished without detectable evidence of disturbance of the animal. This cuff has been used in our laboratory to make controlled observations on the hormonal factors involved in the onset and offset of ascites characterized by renal retention of sodium chloride (18). After constriction of the vena cava, renal retention of sodium is almost immediately apparent and usually becomes maximal within the first twenty-four hours (Figure 2). After release of the constriction, an immediate natriuresis develops in which urine sodium may increase from less than 1 mEq. to over 300 mEq. in the first twenty-four hours. There is an associated diuresis and loss of edema fluid. Similar reversible edema occurs after pulmonary artery constriction, but this is technically more difficult to produce.

In three dogs diabetes insipidus had been induced by Dr. R. C. deBodo of the Department of Pharmacology, New York University College of Medicine, by means of a lesion produced by electrocoagulation in the anterior hypothalamus. Each dog excreted 3 to 6 liters of urine per twenty-four hours when having free access to food and water during the control periods. At the end of the study, the dogs were sacrificed, and the amount of antidiuretic hormone in the hypothalamus and in the pituitary was determined by a method similar to that of van Dyke, Ames, and Plough (14).

RESULTS

Following constriction of the thoracic inferior vena cava to less than half of its original diameter, ascites developed in the two normal control dogs. In both dogs the ascites was massive, requiring the removal of as much as 14 liters of fluid every four to six weeks. In one animal the ascites has persisted for over a year. Balance studies on both dogs (Figure 3) were similar to those previously reported in detail by others (15, 19).

Constriction of the thoracic vena cava in the three dogs with diabetes insipidus was also followed by the development of massive ascites. As indicated by the changes in body weight in two dogs, one diabetic and one with an intact neurohypophysis, presented graphically in Figure 3, ascitic fluid appeared to accumulate at the same rate in both groups following caval constriction. In both, maximal sodium retention developed after caval constriction. For example, the daily urine sodium content fell to a minimum of less than 1 mEq. per day in both studies presented in Figure 3 and never exceeded 8 mEq. per day. Striking differences were observed, however, in the urinary specific gravity and in the daily urine volume. In the dog with diabetes insipidus (Figure 3), urinary specific gravity did not rise above 1.006, whereas in the normal control animal the specific gravity was always above 1.020 and reached a maximum of 1.037. The dog with intact neurohypophysis (Figure 3) excreted small volumes (85 to 425 ml. per day) of urine despite a striking increase in thirst and water intake. In other normal dogs urine volumes as low as 25

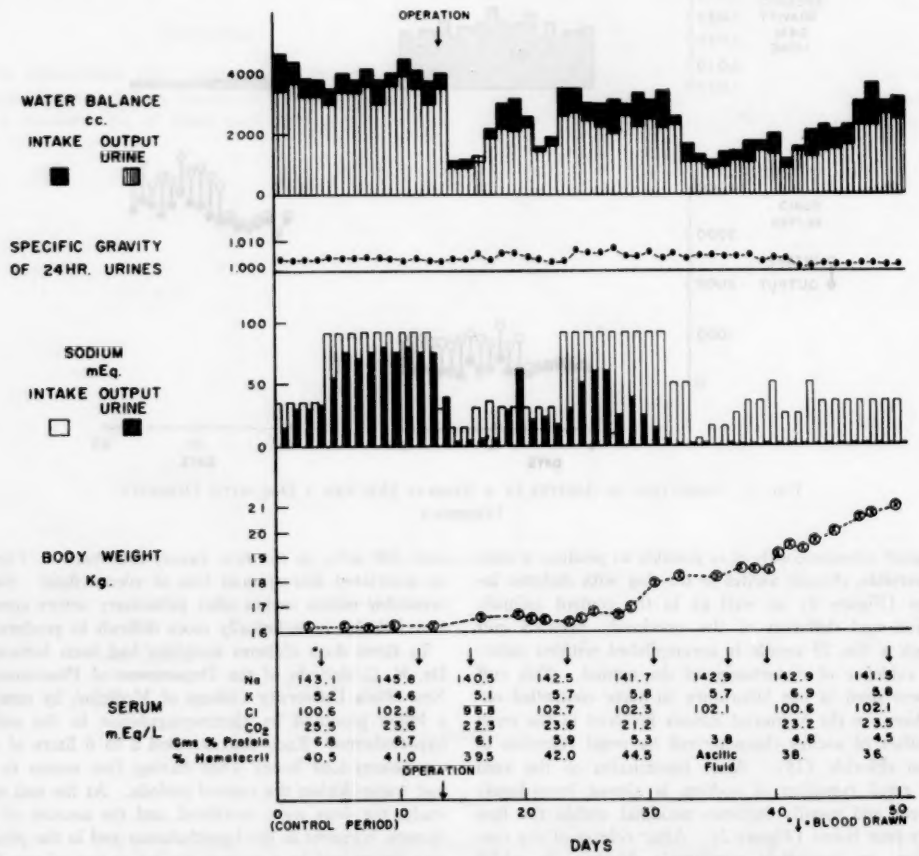

FIG. 4. THE PRODUCTION OF ASCITES IN THE DOG WITH DIABETES INSIPIDUS

ml. per twenty-four hours have at times been noted during ascites formation. In contrast, urinary output increased (3300 to 4000 ml. daily) in association with increased water drinking in the dog with diabetes insipidus during the formation of ascites (Figure 3). Despite the differences observed in water turnover and urine concentration the net water balance and resultant weight gain were comparable in the two dogs. In a second dog with diabetes insipidus, water turnover was reduced slightly during formation of ascites (Figure 4), and in the third animal it did not change appreciably. The control dog (Figure 3), on a constant diet containing 90 mEq. of sodium daily, gained 7.0 kg. in the fifteen days following caval constriction. The diabetic dog received a diet containing 95 mEq. of sodium per day and gained 7.2 kg. in the twelve days following constriction of the thoracic vena cava. These data, therefore, fail to show any significant difference in the rate of formation of ascitic fluid.

More detailed metabolic data for another diabetic dog are presented in Figure 4. This animal gained about 5 kg. of ascites in a period of twenty-five days. Caval constriction was carried out during the operation instead of by subsequent inflation of the cuff. For some reason, urinary sodium retention did not immediately become maximal. The delay could be attributed to an inadequate constriction or perhaps to a post-operative reaction.

The urinary specific gravity in the dogs with diabetes insipidus ranged from 1.001 to 1.010, remaining more or less fixed for a given animal. No appreciable changes in these values were observed during ascites formation. In the diabetic dog presented in Figure 3 a rapid accumulation of the ascitic fluid was evident while the specific gravity of the urine remained constant at 1.004. During this phase of maximal production of ascitic fluid, almost all of the administered sodium was retained. A corresponding reduction in chloride excretion was consistently observed in all animals.

The serum sodium concentration of the diabetic dogs developing ascites remained relatively constant in the normal range. In one of the normal dogs, the serum sodium also remained constant, but in the other control dog forming ascites the serum sodium fell to 138 mEq. per L. from 145 mEq. per L. This tendency to hyponatremia in ascitic dogs has also been observed at times by Davis and Howell (19). Schilling and associates (20) and Davis and Howell (19) have also noted a definite tendency to hyperkalemia in normal dogs with ascites following caval constriction. We have observed a similar change in diabetic dogs with ascites, an example of which is presented in Figure 4. Serum potassium values in excess of 7.0 mEq. per L. were encountered in our animals on five occasions. Periods of rapid edema formation were associated with a striking fall in the serum protein concentration in both the normal and the diabetic dogs. The protein content of the ascitic fluid was found to range from 1.6 to 4.4 gm. per 100 ml. in both groups of animals. Tests of liver function were made in two dogs with ascites, one control and one dog with diabetes insipidus. The protein partition, alkaline phosphatase and serum bilirubin were all found to be well within normal limits, despite enlargement of the liver and gross evidences of cirrhosis after prolonged caval constriction.

TABLE I

The total amount of antidiuretic hormone in the neurohypophysis of the dogs with diabetes insipidus in comparison with normal dogs *

Dog No.	Total mU of ADH: Hypothalamus	Posterior lobe	Total in neurohypophysis
1	67	60	127
2	0	19	19
3	160	725	885
Normal (Av. of 11 dogs)†	4,060	16,700	20,760

* Corresponding metabolic data for dogs 1, 2, 3 are summarized in Figures 2, 3, 4, respectively.
† van Dyke, Adamsons, and Engel (4).

At the time of sacrifice or death, the hypothalamus and the atrophic posterior pituitary of the three dogs with diabetes insipidus were assayed for antidiuretic hormone (Table I). The assays were performed by intravenous injection of tissue extracts into heavily hydrated dogs. Less than 0.5 mU can be detected by this method. Owing to post-operative scarring of the hypothalamus, it was not feasible to assay separately some of the divisions of the neurohypophysis such as the hypothalamico-hypophyseal tract in the tuber cinereum or the paired supraoptic and paraven-

tricular nuclei. The pituitary body was assayed without separation of the lobes. The results in comparison with a group of eleven normal dogs are summarized in Table I. Except in dog No. 3 (Figure 4), only about 4 per cent of the total amount normally present could be detected in the neurohypophysis (hypothalamus and posterior pituitary). Thus, only trivial amounts of stored antidiuretic hormone could be detected in the neurohypophysis of dogs 1 and 2. The reduction in stored hormone was severe but less pronounced in dog 3. The quantities of stored hormone present do not furnish information concerning the rate of formation or liberation of antidiuretic hormone. However, the characteristic polyuria of these animals in association with low or very low neurohypophyseal titers of stored hormone suggests that the formation and liberation of ADH were grossly inadequate.

DISCUSSION

Several groups of investigators have previously characterized the metabolic events accompanying the onset and termination of ascites formation after constriction of the thoracic vena cava (15, 19–21). Such animals exhibit chronic fluid retention with an expanded extracellular fluid volume as a result of more complete reabsorption of sodium by the renal tubules. At least in this important respect, dogs with experimental ascites are similar to patients with cardiac failure and decompensated hepatic cirrhosis. Further studies of Davis, Howell, Southworth, and Laqueur (22, 23) have shown that this type of edema usually clears after adrenalectomy or anterior hypophysectomy. Their data indicate that a salt-retaining hormone of the adrenal is essential for the edema formation and that anterior pituitary activity appears to contribute to ascitic fluid accumulation by maintaining an adequate level of venous hypertension.

The present study clearly demonstrates that ascitic fluid is formed readily in the dog following constriction of the thoracic inferior vena cava with storage of little or very little antidiuretic hormone in the neurohypophysis. Since the very low amounts of stored hormone could be correlated with the excretion of abnormally great volumes of urine, it is presumed that very little antidiuretic hormone could be liberated by the dogs with diabetes insipidus. The ascites of diabetic dogs appears to be similar to the ascites produced in normal dogs, and the rate of its formation was not shown to be appreciably different. In both groups of animals the formation of ascitic fluid is characterized by maximal renal retention of ingested sodium; water and chloride are apparently retained in amounts sufficient to sustain osmolarity of body fluid.

The formation of ascites in dogs with diabetes insipidus appears to differ from that observed in control animals only in that it is characterized by persistence of a dilute urine of large volume. The presence of an intact neurohypophysis would thus appear to account for the comparatively low water turnover noted in the normal controls. It is implied in this, although not established, that overactivity of the antidiuretic hormone could account for oliguria, retention of water in excess of salt and resultant hyponatremia seen at times in the control dogs and in certain patients with ascites.

Animals with diabetes insipidus have an inherently greater tendency to a negative water balance. In this study ascites formation was related to the amount of sodium ingested but was largely independent of water ingested. It is therefore suggested that abnormal retention of sodium and chloride rather than of water is of prime importance in the accumulation of ascitic fluid after constriction of the vena cava.

SUMMARY

Progressive ascites was readily produced by constriction of the inferior vena cava at the level of the diaphragm in two normal control dogs and in three dogs with diabetes insipidus. The diabetes insipidus had been induced previously by bilateral destruction of the neurohypophyseal tracts.

The rate of formation of ascitic fluid after caval constriction was similar in both groups.

At subsequent postmortem little or no antidiuretic hormone could be demonstrated in the neurohypophysis of the diabetic animals.

During ascites formation in normal dogs urine formation tended to decrease, and the urine tended to become more concentrated despite an increase in water intake. On the other hand, among the

diabetic dogs, polydypsia with the continued excretion of large volumes of dilute urine persisted during accumulation of ascitic fluid.

In both groups of animals, the formation of ascites was characterized by nearly maximal renal retention of ingested sodium.

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THE MECHANICS OF BREATHING IN DIFFERENT BODY POSITIONS. I. IN NORMAL SUBJECTS

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(Submitted for publication July 8, 1955; accepted April 16, 1956)

In preliminary experiments some striking differences in the mechanics of breathing in different body positions were observed. In view of those findings it was thought worthwhile to carry out a more extensive study with the anticipation that the results might shed some light on the factors which influence the compliance and mechanical resistance of the lung.

Compliance is defined as the volume change brought about by one centimeter of water pressure (L. per cm. H_2O). The lower the compliance the stiffer the lung and vice versa. Mechanical resistance measures the amount of pressure necessary to obtain a certain flow rate and is expressed as cm. H_2O per L. per sec.

In this paper, mechanical resistance includes both the resistance to air flow *per se* and the resistance to tissue deformation. The latter resistance has been measured to be about 30 to 40 per cent of the total mechanical resistance in normal lungs (1). Because the pressure flow relationships are not linear, the mechanical resistance changes continuously over the respiratory cycle especially in patients with increased resistance. In order to get better comparative values over an entire respiratory cycle, values for mean mechanical resistance are given in this paper.

METHODS

Intraesophageal pressures were substituted for intrapleural pressures. The method used was essentially the one described by Mead and Whittenberger (2, 3), employing an esophageal balloon, 15 cm. in length and inflated with 1 ml. of air. All pressures were measured by mechano-electrical transducers. Flow rates were measured by a pneumotachograph. The volume flow rate was electrically integrated by a condenser-resistor unit. Because the integrator only maintained linearity for 10 seconds, an automatic switch was provided which discharged the condensers at determined time intervals (5 to 8 seconds). A controlled secondary circuit made it possible to correct for volume differences caused by the respiratory quotient and temperature and humidity changes between the inspired and expired air. Consequently the base line remained horizontal in a given midposition. The volumes measured are the inspired volumes at 25°C (not saturated) and are reported as such. This would reduce the compliance values about 7 per cent as compared to values corrected for B.T.P.S. All electrical outputs were fed into a Sanborn four channel recording unit.

Since considerable variations may occur between individual respiratory cycles caused by heart beats, at least two series of ten cycles each were measured in each position and at each respiratory rate. The mean compliance of the two series was required to check within 0.03 for values above 0.15, within 0.02 for values above 0.1 and within 0.01 for values below 0.1. Resistive pressures were measured by drawing the corrected static intrapleural pressure and obtaining the difference between dynamic and static intrapleural pressure at each $\frac{1}{10}$ of a second for slow respiratory rates and at each $\frac{1}{25}$ of a second for rapid rates. The resistive pressure was divided by the flow rate existing at that particular moment and plotted on graph paper—first as pressure against flow and second, as mechanical resistance against time. The mean was measured planimetrically. All pressures were calibrated by a water manometer and flow rates and volumes with suitable Fischer and Porter flowmeters before and after the experiment.

In two subjects with spontaneous pneumothorax intrapleural pressures were measured through an 18-gauge needle and in two patients with pleural effusion with an indwelling 14-gauge polyethylene catheter and a Statham strain gauge whose zero point was the posterior axillary line.

The studies were done on eight subjects who by history, clinical, and x-ray findings were free of chronic pul-

TABLE I

Physical characteristics of eight normal subjects

Patient	Sex	Age	B.S. (m^2)	V.C. (erect) (ml.)	M.B.C. (L./min.)
B. D.	M	35	1.84	4,300	115
W. C.	M	37	1.70	3,500	
J. R.	F	21	1.56	2,900	96
M. G.	M	30	1.80	4,500	140
J. T.	M	23	1.86	4,000	
C. B.	M	37	1.78	3,600	
J. C.	M	49	1.65	3,835	128
H. T.	M	20	1.65	2,200	80

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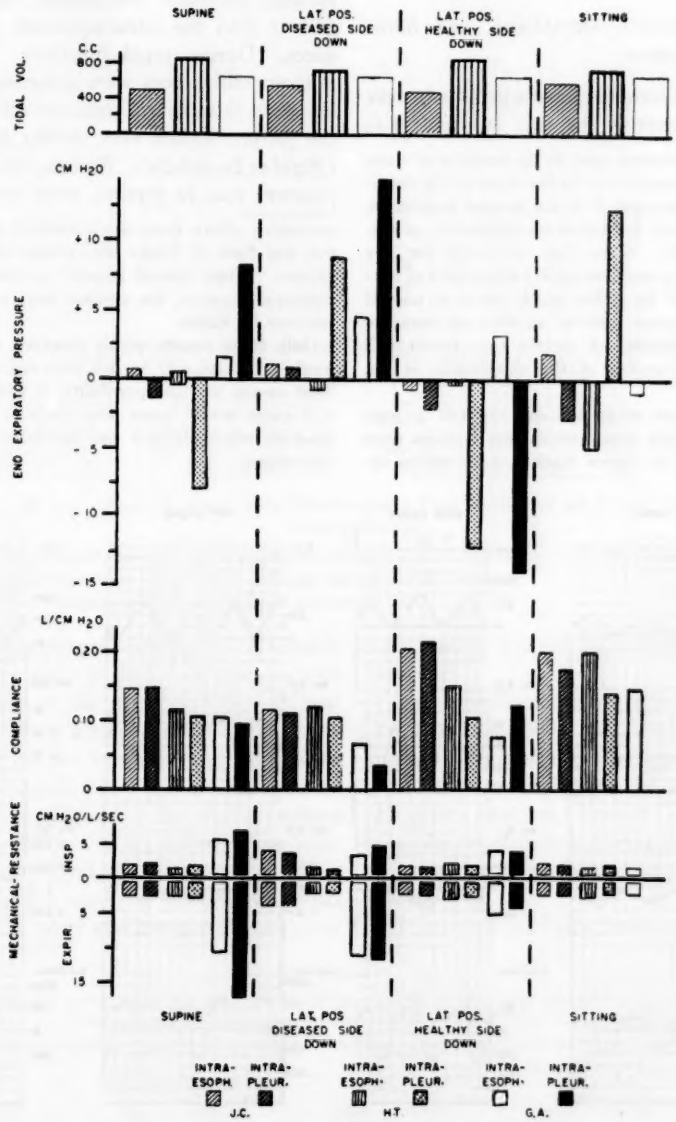
monary disease. Their physical characteristics are given in Table I. One of the subjects (M. G.) was studied twice. Two of those subjects (J. C. and H. T.) had a spontaneous pneumothorax, and intrapleural and intraesophageal pressures were measured simultaneously. In addition, intrapleural and intraesophageal pressures were compared in two patients with pleural effusion. The positions studied by intraesophageal pressure were the



THE MECHANICS OF BREATHING IN DIFFERENT BODY POSITIONS. COMPARISON BETWEEN INTRA-ESOPHAGEAL AND INTRA-PLEURAL MEASUREMENTS IN TWO PATIENTS WITH SPONTANEOUS PNEUMOTHORAX (J.C. AND H.T.) AND ONE PATIENT WITH PLEURAL EFFUSION (G.A.)

FIGURE 1

supine in nine experiments, the sitting in nine experiments and the prone in six experiments. Most positions were studied during slow and fast respiratory rates.

RESULTS [2]

1. *Comparison between intrapleural and intraesophageal pressures*

Intrapleural and intraesophageal pressures were measured simultaneously in two subjects with spontaneous pneumothorax (10 per cent collapse) and in two patients with pleural effusion. The results in three of these patients during slow breathing are shown in Figure 1. In all cases, the intrapleural pressure differences were somewhat greater than the intraesophageal pressure differences. During rapid breathing, intraesophageal pressure differences were sometimes considerably less than intrapleural pressure differences though the pattern looked very similar in both tracings (Figures 2A and 2B). End expiratory intrapleural pressure was, in general, more negative than end

[2] The statistical technique used in the analysis of these results makes no assumption as to the form of the distribution of the variables studied in the general population. Such statistical methods are called nonparametric or distribution-free methods. Since they are valid for any parent population, they could be applied to samples of normal population. On the other hand, using a normal theory test with a given level of significance does not assure that the probability of certain type errors will not occur if the normality of the distribution is not known.

If comparisons were made between any two groups, rates, positions or their combinations, the findings were treated according to the signed Rank test for paired observations where there were matched pairs or the Sign test and Sum of Ranks test, where there were unequal groups. When several groups, positions or rates were compared together, the method used was an analysis of variance by Ranks.

Only those results which occurred at the 5 per cent level of confidence or beyond were reported as significant. That means that the possibility of whatever comparison was made would occur five times or less out of one hundred, which places it well beyond the range of chance occurrence.

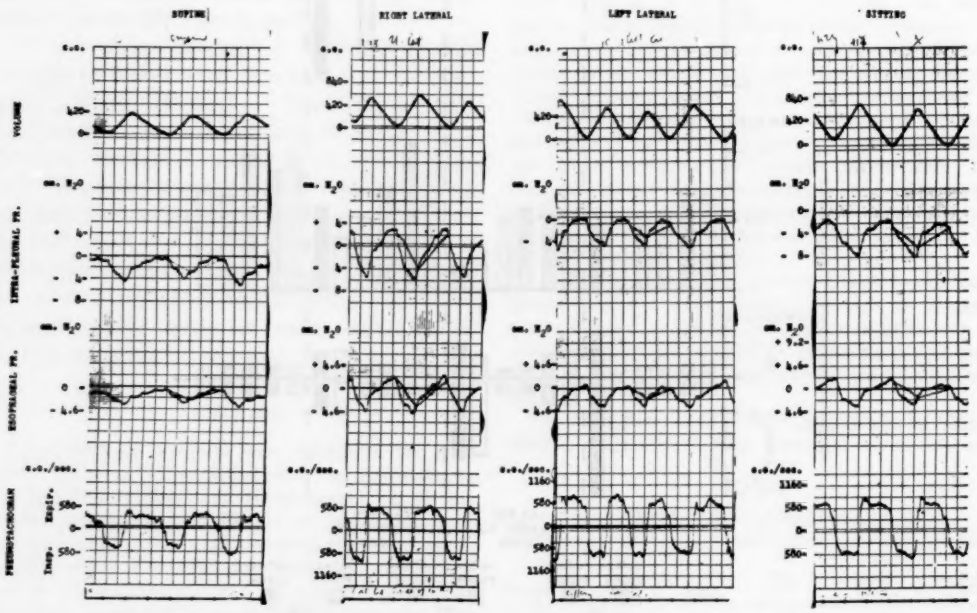


FIG. 2A. J. C., 49 YRS., MALE; NORMAL WITH SPONTANEOUS PNEUMOTHORAX ON RIGHT SIDE WITH 10 PER CENT COLLAPSE

Comparison of intrapleural and intraesophageal pressures in a normal patient with spontaneous pneumothorax on the right side with 10 per cent collapse. During quiet breathing, the pressure difference in the intrapleural and esophageal tracings are nearly the same although the absolute pressure levels are slightly higher in the intrapleural tracings depending upon position.

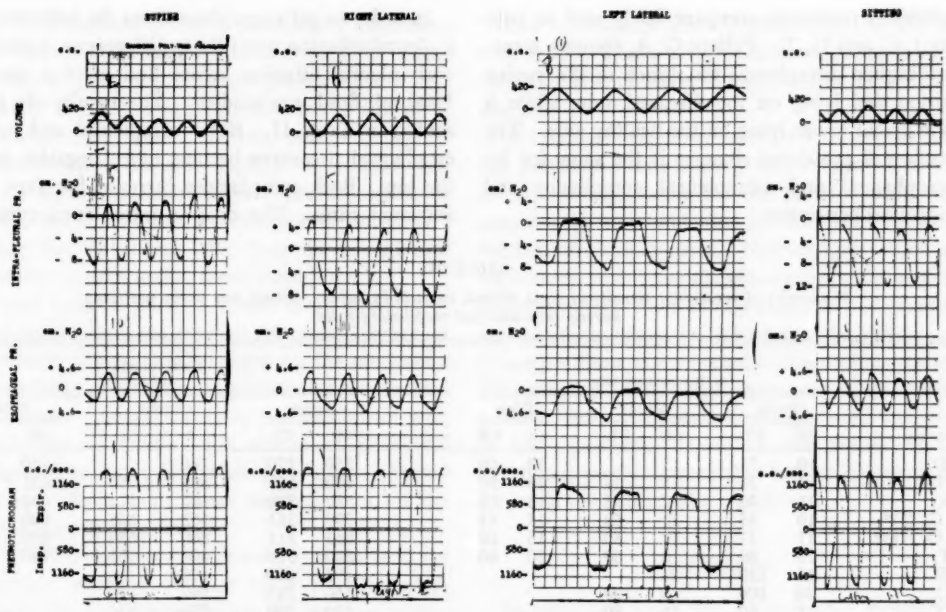


FIG. 2B. J. C., 49 YRS., MALE; NORMAL WITH SPONTANEOUS PNEUMOTHORAX ON RIGHT SIDE WITH 10 PER CENT COLLAPSE

Comparison of intrapleural and intraesophageal pressures in a normal patient with spontaneous pneumothorax on the right side with 10 per cent collapse during rapid breathing.

Note the marked dampening in the esophageal pressure.

expiratory intraesophageal pressure. A notable exception was the study in the lateral position with the patients lying on the diseased lung. Patient G. A. shows a higher intrapleural pressure in the supine position as the result of the hydrostatic pressure of the fluid. No reliable intrapleural pressures could be obtained in the sitting position on this patient. Subject H. T. showed a considerably higher intrapleural pressure in the sitting position. He had a small bronchopleural fistula at the time of the study and his intrapleural pressure tracing showed light damping in the sitting position.

The compliance values compare well for subject J. C. in all positions. Subject H. T. has a reduction in intrapleural as compared to intraesophageal compliance lying on the healthy side and in the sitting position. Patient G. A. shows a lower intrapleural compliance lying on the diseased side and a higher intrapleural compliance lying on the healthy side.

The values for intrapleural and intraesophageal

TABLE II

Mechanics of breathing in patient A. A., 65 years, male, with pleural effusion on the right side (Study in supine position before and after removal of 1,000 ml. of fluid)

Respiratory rate	Tidal volume	End expir. pressure		Compliance		Mean mech. resist. during insp.		Mean mech. resist. during expir.	
		Intra pl. pr.	Esoph. pr.	Intra pl. pr.	Esoph. pr.	Intra pl. pr.	Esoph. pr.	Intra pl. pr.	Esoph. pr.
Before									
24	560	+12.0	+2.5	0.08	0.087	5.40	4.75	9.25	8.80
60	500	+12.0	+3.0	0.052	0.072	6.75	5.25	5.70	5.50
After									
24	450	+ 1.0	−2.0	0.12	0.128	2.60	4.30	5.10	5.30
88	280	− 2.5	−5.2	0.043	0.097	3.50	3.30	2.70	3.15

mechanical resistance compare fairly well in subjects J. C. and H. T. Patient G. A. shows a somewhat higher intrapleural resistance in the supine position and lying on the diseased side, while it is somewhat lower lying on the healthy side. The direction of positional changes is the same for intraesophageal and intrapleural compliance and mechanical resistance.

In order to get some ideas about the influence of a pleural effusion upon these differences, a patient with pleural effusion before and after a tap of 1000 ml. fluid was studied. The results are presented in Table II. Both intrapleural and intraesophageal pressures become more negative after the tap. Both compliances increase for slow respiratory rates. The drop in intrapleural compli-

TABLE III

Mechanics of breathing: Results in eight normal subjects in supine, sitting and prone positions during slow and fast respiratory rates

	Respiratory rate						Tidal volume (*ml.*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
B. D.	10	75	13		12	60	750	570	740		740	690
W. C.	15	33	15	44	20	50	490	850	505	1,050	607	951
J. R.	15	70	12	75	12	75	580	200	463	370	694	405
M. G. (I)*	10	55	22	50	15	43	700	545	665	655	800	700
M. G. (II)†	11	37	17	50	15	40	845	811	800	650	830	820
J. T.	25	60	16	60	30	60	300	570	400	355	430	650
C. B.	15	120	24				496	600	570			
J. C.	20	100	6	100			560	245	592	270		
H. T.	11	80	14	80			850	350	750	435		
Mean	14.7	70	15.5	66	17.3	55	620	528	610	542	682	702

	Intraesophageal end expiratory pressure (*cm. H_2O*)						Compliance (*L./cm. H_2O*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
B. D.	+2.0	+1.4	+0.25		+ 2.8	+ 0.6	0.125	0.120	0.240		0.136	0.146
W. C.	+0.5	+1.75	0	+2.2	− 0.25	+ 1.4	0.223	0.225	0.205	0.225	0.150	0.162
J. R.	+2.0	+0.5	−2.2	−3.5	+ 0.5	− 2.2	0.105	0.082	0.177	0.169	0.189	0.200
M. G. (I)*	+0.5	−0.6	−5.0	−3.5	−13.0	−10.0	0.140	0.150	0.162	0.144	0.072	0.073
M. G. (II)†	+1.25	+2.6	−3.5	−4.5	− 7.5	− 6.0	0.147	0.146	0.175	0.160	0.097	0.117
J. T.	+0.5	−2.5	0	−1.0	− 2.8	− 5.5	0.128	0.090	0.253	0.262	0.230	0.205
C. B.	+1.0	+1.0	−3.5				0.107	0.085	0.174			
J. C.	+1.8	+0.9	+1.1	−2.0			0.143	0.162	0.161	0.161		
H. T.	0	−1.0	−5.0	−5.0			0.112	0.112	0.200	0.210		
Mean	+1.06	+0.66	−2.0	−2.5	− 3.4	− 3.6	0.137	0.130	0.195	0.190	0.146	0.150

	Mean mechanical resistance during inspiration (*cm. H_2O/L./sec.*)						Mean mechanical resistance during expiration (*cm. H_2O/L./sec.*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
B. D.	2.70‡	1.60	1.34		0.40‡	0.65	1.62	1.72	1.55		1.20	0.85
W. C.	0.68		0.65	0.24	1.00		1.30		2.74	1.00	3.00	
J. R.	5.40	5.00	3.90	4.20	3.80	3.20	7.30	5.30	4.90	5.20	4.00	3.50
M. G. (I)*	3.00	2.67	2.25	3.20	2.30	2.80	4.35	2.22	2.60	3.30	4.40	3.00
M. G. (II)†	3.05	3.30	2.30	2.90	1.60	2.23	4.25	3.85	3.50	3.20	3.60	3.40
J. T.	6.50	3.60	1.90	2.10	3.70	3.54	6.60	3.90	2.70	2.50	4.10	5.10
C. B.	3.30	5.80	1.47				3.40	5.10	2.30			
J. C.	2.18	2.83	2.34	2.60			2.38	2.80	2.32	2.22		
H. T.	1.50	2.40	1.65	1.00			2.50	3.00	2.70	1.30		
Mean	3.14	3.40	1.98	2.32	2.13	2.48	3.74	3.49	2.81	2.68	3.38	3.17

* First study.
† Second study—two months later.
‡ Marked interference of heart beats.

ance with increase in rate is particularly noticeable after the pleural tap, while the drop in intraesophageal compliance though present is less significant. Intraesophageal mechanical resistance is somewhat lower before the tap, both for inspiration and expiration, and slightly higher after the tap for slow respiratory rates. Corresponding to the marked drop noted in intrapleural compliance during fast breathing, the drop in intrapleural resistance is less under these conditions.

From these results, it would seem that intraesophageal pressures give an adequate picture of the effects of positional changes on the mechanics in breathing. However, in patients with unilateral dysfunction (pleural effusion, penumothorax with bronchopleural fistula) the effects of positional changes on the intrapleural pressures of the affected lung are considerably more marked than on the intraesophageal pressures.

The difference between intraesophageal and intrapleural pressure depends upon the characteristics of the measuring device, its location within the esophagus and the characteristics of the esophageal wall and the adjacent structures (2–5). Difficulties may ensue in employing intraesophageal pressures as a guide for the determination of absolute lung distention. Therefore, an evaluation of the work of breathing using intraesophageal pressure measurements will not take into account the metabolic work necessary to maintain the inflation of the lung at a given midposition.

2. Positional changes

The results for the three positions at two different respiratory rates are presented in Table III.

End expiratory pressures are consistently more negative in the sitting than in the supine position. In four out of six normals, they were more negative in the prone than in the sitting position. These differences are significant at the 2 per cent level. There are no significant differences in end-expiratory pressures between slow and fast respiratory rates in any position. In general, subjects who show a decrease in tidal volume with an increase in respiratory rate, demonstrate an increase in negativity.

Compliance values are considerably higher in the sitting than in the supine position. The values in the prone position are usually intermediate with considerable scattering of the individual scores. These differences are significant at the 2.5 per cent level. There are no differences for compliance values at different rates in any of the positions studied.

Mechanical resistance is somewhat higher during expiration than during inspiration. The difference is significant for all positions for slow respiratory rates and also for the prone position for fast respiratory rates. Neither inspiratory nor expiratory resistance change significantly at various respiratory rates in any position. Mechanical resistance is generally lower in the sitting and prone positions as compared to the supine position. The subjects with a high resistance in the supine position show the greatest changes with variation of body position. However, the scatter of the individual results is such that the difference of the means is not statistically significant.

DISCUSSION

In changing from the erect to the recumbent position there is, in general, only a slight decrease in total lung capacity, vital capacity, and residual volume but a large decrease in expiratory reserve volume with an inadequate compensatory increase in the inspiratory reserve volume (6–10). Pulmonary mixing is poorer in the supine than in the erect position (11–18). In the lateral position the lower lung shows a decreased expiratory reserve volume. However, the oxygen consumption, minute ventilation, vital capacity, and inspiratory reserve volume are increased (19). During thoracic surgery, there is considerably more carbon dioxide retention in the lateral position than in the supine or prone position (20).

The pressure-volume curves of the relaxed thorax (relaxation curves) are parallel in the sitting, supine and Trendelenburg positions (21, 22). The relaxation volume of the thorax is nearer the expiratory volume in the recumbent and Trendelenburg positions. Under these conditions the lung assumes a lower midposition. In the erect position, the weight of the thorax tends to increase the intrathoracic pressure whereas the pull of the abdominal contents tends to decrease it. Judging from the relaxation curves, the pull of the abdominal contents seems to predominate.

Although respiratory rates and tidal volumes in the different body positions were within the same

range in the experiments (only the prone position showed a considerable increase in the minute ventilation), changes in body position resulted in considerable changes of end expiratory pressure, compliance, and mechanical resistance. The changes in end expiratory pressures are greater than one would expect from changes in midposition alone (23, 24). If the observed changes in compliance were only the result of changes in midposition, they should also have been noted in changing from slow to rapid respiratory rates, where marked changes in tidal volumes and midposition occurred. Therefore, one has to look for additional factors which could contribute to the observed changes in the mechanics of breathing.

A decrease in hydrostatic pressure by removal of intrapleural fluid leads to an increase in compliance and a decrease in mechanical resistance. Changes in hydrostatic pressure could influence the physical properties of the lung directly, but they also can affect both the distribution of pulmonary blood flow and the forces acting upon the chest wall, mediastinum and diaphragm. The latter in turn changes the shape of the thorax and, therefore, the midposition of the lung.

The amount and distribution of blood within the lungs influences pulmonary surface tension and, therefore, compliance and mechanical resistance. In patients with mitral stenosis (25) studied in the sitting position, the deviation from normal values was more marked when the patients were in failure. Some of the changes observed in the supine position may be the result of increased thoracic blood volume, thus simulating to some extent the changes observed in mitral stenosis. Oser, Ruston, and Ryan (26) showed that the increase in venous hydrostatic pressure in the erect position can exceed the venous tone below the diaphragm and result in blood trapping and reduction of right atrial pressure.

Another factor to be considered is the amount of unequal ventilation and the opening up of additional ventilatory space when the lung volume is increased (27). Our results seem to indicate that this is a minor factor in normal lungs, compliance being constant at different respiratory rates (28).

The reported changes in the mechanics of breathing are probably of no practical importance in normal subjects who have a large functional reserve and are capable of compensating automatically for the occurring changes. However, the problem is quite different for patients with cardiopulmonary disease. Even for the normal subject, it is important to report results of pulmonary function tests with the body position in which the patient was studied. The changes with body position can exceed the variation of normal values in a given position.

SUMMARY

1. The mechanics of breathing were studied in eight normal subjects.

2. Intrapleural and intraesophageal pressures were compared in four patients in different body positions. Intrapleural pressure differences were usually somewhat greater than intraesophageal pressures. End expiratory intraesophageal pressure is usually more positive than end expiratory intrapleural pressure. The difference varies from subject to subject and in each depending upon the body position. Intraesophageal pressures seem to be adequate for the measurement of the mechanics of breathing, but unsuitable for the determination of absolute lung distention (midposition).

3. Compliance and mechanical resistance were measured during slow and rapid breathing in the supine, sitting and prone positions.

4. Compliance was lowest in the supine and highest in the sitting position and did not change with change in respiratory rate.

5. Mechanical resistance was usually highest in the supine and lowest in the sitting position, expiratory resistance being somewhat higher than inspiratory resistance in all positions studied.

6. Some of the physiologic factors which might contribute to the observed changes are discussed.

7. The full significance of pulmonary function testing can only be determined after considering the body position during the tests.

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THE MECHANICS OF BREATHING IN DIFFERENT BODY POSITIONS. II. IN CARDIOPULMONARY DISEASE

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(Submitted for publication July 8, 1955; accepted April 16, 1956)

In a previous paper, the effects of changes in body position upon the mechanics of breathing in normals were reported (1). This report deals with the results obtained in two groups of patients studied in the supine, sitting, prone, head down and lateral positions.

MATERIAL AND METHODS

The technic described in the previous paper has been employed throughout the study. It consists essentially of measuring intraesophageal pressures, air flow rates and volume changes simultaneously and continuously throughout the respiratory cycle. From these measurements, compliance (volume change per unit pressure change expressed as L. per cm. H_2O) and mechanical resistance (pressure necessary to obtain a given flow rate, expressed in cm. per L. per sec.) have been determined in the different body positions and at different respiratory rates. Mechanical resistance is reported as the mean mechanical resistance of the inspiratory and expiratory phases of the respiratory cycle. In addition, vital capacities were determined with a Benedict-Roth type spirometer, maximal breathing capacities with a low resistance Rudolph valve and a Douglas bag during a 15-second period, and residual volume and intrapulmonary mixing by Darling's seven-minute nitrogen washout method. These tests were carried out in the sitting position. Arterial blood gases, obtained from the brachial artery, were determined with the van Slyke technic, pH with a Cambridge Research Model pH Meter and pCO_2 by Hasting and Singer's Nomogram.

The first group of patients consisted of twelve subjects whose predominant disease was chronic pulmonary emphysema. The second group was composed of various cardiopulmonary diseases. Each patient was studied in at least two positions at slow and rapid respiratory rates. The rates were selected according to the condition of the patient. The physical characteristics of the patients together with their diagnosis and the results of routine pulmonary function tests appear in Tables Ia and Ib. Patients M. N. and H. P. were studied on two separate occasions during different degrees of disability.

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RESULTS [2]

The results are presented in Tables II, III, IV and V.

1. *Patients with chronic pulmonary emphysema studied in the supine, sitting and prone positions*

The *end expiratory pressures* are as a rule highest in the supine position and lowest in the sitting position. However, because of the considerable variation of the individual results, the difference of the means is not statistically significant.

Compliance values are lower in the supine and sitting positions as compared to the previously reported normal group whereas the values for the prone position are generally higher. With the exception of the prone position, the differences of the means are significant at the 1 per cent level. The compliance is lowest in the supine position, rises considerably in the sitting position and remains high in the prone position. These differences are significant at the 5 per cent level. In all positions, there is a marked decrease of compliance with increase in the respiratory rate; the greatest drop occurs in the supine position.

Mechanical resistance is significantly higher during expiration than during inspiration in all positions and at all respiratory rates. Both expiratory and inspiratory resistances are highest in the supine position and show a considerable drop in the sitting position. The values in the prone position are usually between these two extremes. There is no significant difference between the resistance values in a given position at different respiratory rates. As compared to the normal group, inspiratory resistance is, on the average, in-

[2] The statistical analysis has been carried out by the methods described in the first paper (1).

TABLE Ia

Patients with chronic pulmonary emphysema

Patient	Diagnosis	Sex	Age	B.S. (m^2)	V.C. (*ml.*)	M.B.C. (*L./min.*)	RV/TC (%)	Intra-pulmonary mixing (N_2 %)	Arterial blood		
									pH	O_2 sat. (%)	pCO_2
N. S.	Chronic pulmonary emphysema, secondary polycythemia	M	64	1.85	3,000				7.39	85.2	54.0
A. A.	Chronic pulmonary emphysema	M	59	1.70	2,000	54.0			7.39	84.3	40.0
W. L.	Chronic pulmonary emphysema	M	49	1.99	1,900		65	13.0	7.42	76.5	50.0
J. S.	Chronic pulmonary emphysema, hypertension	M	55	1.77	3,410	61.0	53	7.75	7.41	83.9	44.0
M. P.	Chronic pulmonary emphysema, respiratory acidosis	M	64	1.72	3,000	49.0			7.28	67.7	60.0
A. R.	Chronic pulmonary emphysema, cor pulmonale	M	61	1.53	3,320	32.0	57	14.1	7.40	86.5	43.0
W. G.	Chronic pulmonary emphysema, cor pulmonale	M	61	2.07	2,690	49.6	57	3.1	7.39	88.5	35.5
A. B.	Chronic pulmonary emphysema, kyphoscoliosis, polycythemia	M	61	1.76	3,170	100.0	46	6.0	7.40	85.7	43.0
M. N. (I)	Chronic pulmonary emphysema, chronic bronchitis	M	50	1.68	2,955	51.2	64	9.7	7.40	86.0	44.0
M. N. (II)	Same as above	M	50	1.68	2,200	47.0					
M. S.	Chronic pulmonary emphysema	M	48	1.74	4,230	115.0	39	2.2			
J. S.	Chronic pulmonary emphysema	M	48	1.61	2,340	50.0	48	11.7	7.40	87.7	44.0

TABLE Ib

Patients with various cardiopulmonary diseases

Patient	Diagnosis	Sex	Age	B.S. (m^2)	V.C. (*ml.*)	M.B.C. (*L./min.*)	RV/TC (%)	Intra-pulmonary mixing (N_2 %)	Arterial blood		
									pH	O_2 sat. (%)	pCO_2
R. W.	Chronic bronchial asthma	M	35	1.32	4,400						
H. P. (I)	Chronic bronchial asthma, chronic pulmonary emphysema	M	16	1.85	3,600	114.0			7.37	94.5	41.5
H. P. (II)	Same as above	M	16	1.85	2,500	73.0	60	6.0			
M. G.	Chronic bronchial asthma, chronic pulmonary emphysema	F	31	1.82	2,700	43.3	47.4	3.8	7.40	92.0	35.0
A. DeW.	Scleroderma	F	51	1.56	2,300	58.0	49	6.2	7.42	97.2	39.0
B. B.	Left pneumonectomy for bronchial carcinoma	M	65	1.67	2,500	35.0	50	13.5	7.38	82.1	50.0
E. J.	Aneurysm of aorta with atelectasis of left lung	M	63	1.72	1,530	52.0	56	19.6	7.41	81.4	39.0
W. F.	Calcified right pleura	M	73	1.56	2,760	66.0	47	3.46	7.39	88.6	37.0
G. A.	Pleurisy left	M	56	1.97	2,700	114.0			7.37	94.3	40.5
A. A.	Cardiac failure, pleural effusion right	M	65	1.53	2,500	46.7				92.5	
J. Q.	Mitral stenosis	M	23	1.76	4,700	132.2			7.39	98.1	39.0

TABLE II

Mechanics of breathing: Results in twelve patients with chronic pulmonary emphysema in supine, sitting and prone positions during slow and fast respiratory rates

	Respiratory rate						Tidal volume (*ml.*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
N. S.	24	40	24				460	840	520			
A. A.	15		18				460		560			
W. L.	15	33	17	30			550	360	615	490		
J. S.	27	100	27	95	30	100	486	150	600	200	585	220
M. P.	22	60	22	75	22	75	700	370	775	400	875	300
A. R.	15	55	20	36	20	40	630	280	490	350	705	390
W. G.	20	55	22	80	27	76	770	550	770	420	1,060	540
A. B.	17	85	24	75	22	54	650	510	615	530	675	590
M. N. (I)*	18	75	20	75	22	75	550	285	565	335	530	350
M. N. (II)†	18	95	16	80	18	94	730	325	876	454	800	400
M. S.	23	125	24	185			460	900	450	870		
J. S.	17	80	24	68	24	80	430	320	520	380	500	290
Mean	19.3	73	21.5	80	23	74	575	445	615	465	715	385

	Intraesophageal end expiratory pressure (*cm. H_2O*)						Compliance (*L./cm. H_2O*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
N. S.	−2.0	−0.7	−4.5				0.072	0.064	0.110			
A. A.	−1.5		−1.2				0.110		0.120			
W. L.	0	+4.4	−7.0	−7.0			0.029	0.018	0.075	0.034		
J. S.	−5.4	−4.0	−2.2	−3.0	−6.9	−4.6	0.072	0.022	0.134	0.061	0.134	0.078
M. P.	−0.5	−3.0	−4.0	−5.0	−6.5	−7.0	0.148	0.090	0.270	0.155	0.270	0.180
A. R.	+0.2	−0.1	−2.0	−0.4	0	−2.1	0.139	0.050	0.195	0.110	0.233	0.163
W. G.	−0.4	−0.6	−2.7	−3.3	−0.3	+0.8	0.132	0.095	0.170	0.154	0.235	0.088
A. B.	−1.6	−4.5	−5.0	−4.4	−6.3	−7.0	0.092	0.072	0.100	0.092	0.090	0.081
M. N. (I)*	−3.2	−2.3	−4.8	−5.4	−4.0	−4.5	0.124	0.078	0.215	0.165	0.262	0.173
M. N. (II)†	+0.2	−7.5	−1.75	0	−0.2	+1.7	0.111	0.040	0.186	0.062	0.158	0.052
M. S.	+0.25	+3.0	−6.0	−2.5			0.118	0.065	0.196	0.066		
J. S.	−2.0	+2.7	+2.0	−0.2	−2.0	−3.8	0.067	0.025	0.110	0.064	0.068	0.044
Mean	−1.75	−0.5	−3.3	−2.9	−3.3	−3.32	0.101	0.056	0.157	0.096	0.181	0.108

	Mean mechanical resistance during inspiration (*cm. H_2O/L./sec.*)						Mean mechanical resistance during expiration (*cm. H_2O/L./sec.*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
N. S.	9.00	6.10	5.10				21.20	8.30	4.67			
A. A.	6.95		4.35				7.85		6.10			
W. L.	38.80	29.00	16.60	24.00			41.75	76.00	34.20	31.50		
J. S.	10.10	10.30	4.90	4.00	3.65	3.85	48.00	27.00	11.78	12.20	9.00	7.77
M. P.	6.80	10.70	7.30	7.50	7.90	12.00	13.40	15.85	10.00	14.20	14.70	16.90
A. R.	10.20	8.90	5.20	6.36	10.50	7.30	15.15	14.50	13.90	11.55	19.65	11.10
W. G.	9.05	7.20	7.60	6.95	5.40	4.95	9.00	20.00	10.90	11.90	5.45	3.70
A. B.	5.33	3.48	3.22	2.86	2.43	2.23	9.60	4.90	4.11	2.94	5.19	3.35
M. N. (I)*	9.36	8.75	4.90	3.60	4.40	4.40	13.80	8.40	8.80	4.55	6.44	5.65
M. N. (II)†	6.60	5.65	6.70	3.45	5.65	3.45	9.50	7.75	6.00	7.23	6.45	5.55
M. S.	4.20	3.10	2.20	2.16			3.85	5.60	2.10	2.25		
J. S.	8.00	8.25	4.75	3.65	5.95	6.20	8.00	17.80	3.20	5.40	6.70	16.00
Mean	10.40	9.20	6.05	6.45	5.75	5.55	16.80	18.75	9.65	10.40	9.20	8.75

* First study.
† Second study—two months later.

creased three times and expiratory resistance five times (significant at 0.1 per cent level). Similar differences between normal subjects and patients with chronic pulmonary emphysema have been reported by others (2–6).

2. Patients with various cardiovascular diseases (studied in supine, sitting and prone positions)

Compliance values are significantly lower and resistance values significantly higher than in the normal group. Even though this group covers a

TABLE III

Mechanics of breathing: Results in patients with various cardiovascular diseases in supine, sitting and prone positions during slow and fast respiratory rates

	Respiratory rate						Tidal volume (*ml.*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
R. W.	15	68	13	60	19	65	710	300	960	545	870	385
A. DeW.	20	94	17	107	15	100	400	375	570	485	423	338
E. J.	30	43	25	75	33	50	490	450	510	384	530	528
B. B.	20	50	30	70			520	380	490	330		
W. F.	16	65	17	71			610	450	410	550		
G. A.	11	80	14	80			670	330	650	350		
H. P. (I)*	17	90	18	80			550	400	650	600		
H. P. (II)†	22	100	20	115			490	220	490	370		
M. G.	11	55	11	79			780	385	820	360		
J. Q.	10	65	15	60			475	950	656	960		
Mean	17.2	71	18	80	22.3	71.6	570	425	620	493	604	417

	Intraesophageal end expiratory pressure (*cm. H_2O*)						Compliance (*L./cm. H_2O*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
R. W.	+1.5	−2.0	− 2.4	− 5.5	−3.8	−5.5	0.110	0.068	0.150	0.130	0.130	0.100
A. DeW.	+1.5	+5.5	+ 3.0	+12.0	+1.0	+5.1	0.052	0.025	0.083	0.021	0.092	0.024
E. J.	−1.0	+1.0	− 5.5	− 5.7	−5.0	−5.3	0.058	0.057	0.060	0.056	0.068	0.056
B. B.	−2.0	−6.2	− 3.2	− 8.5			0.045	0.029	0.060	0.031		
W. F.	−0.8	+4.0	− 9.0	− 7.8			0.145	0.080	0.086	0.104		
G. A.	+1.5	+1.0	− 1.5	− 2.5			0.105	0.084	0.142	0.141		
H. P. (I)*	−5.5	−6.7	− 5.0	− 4.5			0.165	0.133	0.202	0.224		
H. P. (II)†	−2.5	−4.5	− 6.0	− 6.0			0.090	0.047	0.125	0.075		
M. G.	+0.6	−2.0	− 1.5	− 1.2			0.107	0.058	0.136	0.055		
J. Q.	−2.0	+2.0	−10.0	− 8.0			0.076	0.070	0.107	0.118		
Mean	−0.9	−0.8	− 4.1	− 3.8	−2.6	−1.9	0.095	0.065	0.115	0.096	0.097	0.060

	Mean mechanical resistance during inspiration (*cm. H_2O/L./sec.*)						Mean mechanical resistance during expiration (*cm. H_2O/L./sec.*)					
	Supine		Sitting		Prone		Supine		Sitting		Prone	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
R. W.	7.25	6.80	4.50	5.40	6.35	7.90	7.25	6.90	6.80	5.30	6.40	8.30
A. DeW.	12.20	6.50	10.30	8.80	22.80	10.10	19.30	9.80	12.50	12.10	14.00	10.10
E. J.	6.80	8.90	5.20	3.60	4.70	4.90	10.65	9.90	8.40	5.80	6.15	7.30
B. B.	13.00	12.50	9.20	8.90			13.60	17.00	8.00	9.70		
W. F.	3.18	2.41	2.20	3.14			6.80	4.46	3.45	3.27		
G. A.	5.70	3.10	1.75	1.84			10.60	5.75	2.06	2.63		
H. P. (I)*	3.25	3.65	2.80	1.74			4.25	3.30	2.50	1.62		
H. P. (II)†	7.20	5.70	3.90	3.70			9.65	8.45	5.65	4.40		
M. G.	10.90	8.90	12.40	9.50			21.80	12.00	17.50	17.00		
J. Q.	6.70	3.70	5.55	3.10			9.00	4.25	7.90	4.10		
Mean	7.62	6.22	5.78	4.97	11.30	7.63	11.29	8.18	7.48	6.59	8.82	8.57

* First study.
† Second study—one month later.

TABLE IV

Mechanics of breathing: Results in five patients in supine, sitting and head down positions during slow and fast respiratory rates

	Respiratory rate						Tidal volume (*ml.*)					
	Supine		Sitting		Head down		Supine		Sitting		Head down	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
M. S.	23	125	24	185	20	150	460	900	450	870	520	685
M. N. (II)	18	95	16	80	20	100	730	325	867	454	650	325
H. P. (II)	22	100	20	115	24	80	490	220	490	370	585	330
M. G.	11	55	11	79	15	83	780	385	820	360	1,000	320
J. Q.	10	65	15	60	10	70	475	950	655	960	730	1,130
Mean	16.8	88	17.2	104	17.8	96.6	587	556	656	603	698	558

	Intraesophageal end expiratory pressure (*cm. H_2O*)						Compliance (*L./cm. H_2O*)					
	Supine		Sitting		Head down		Supine		Sitting		Head down	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
M. S.	+0.25	+3.00	− 6.00	−2.50	+0.50	+1.50	0.118	0.065	0.196	0.066	0.231	0.050
M. N. (II)	+0.20	−0.75	− 1.75	0	+1.40	+1.10	0.111	0.040	0.186	0.062	0.105	0.040
H. P. (II)	−2.50	−4.50	− 6.00	−6.00	−1.00	0	0.090	0.047	0.125	0.075	0.095	0.052
M. G.	+0.60	−2.00	− 1.50	−1.20	+1.20	−1.20	0.107	0.058	0.136	0.055	0.103	0.041
J. Q.	−2.00	+2.00	−10.00	−8.00	+1.50	+6.00	0.076	0.070	0.107	0.118	0.086	0.062
Mean	−0.70	−0.45	− 5.00	−3.50	+0.70	+1.50	0.100	0.056	0.150	0.075	0.124	0.049

	Mean mechanical resistance during inspiration (*cm. H_2O/L./sec.*)						Mean mechanical resistance during expiration (*cm. H_2O/L./sec.*)					
	Supine		Sitting		Head down		Supine		Sitting		Head down	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
M. S.	4.20	3.10	2.20	2.16	2.95	1.75	3.85	5.60	2.10	2.25	2.20	2.15
M. N. (II)	6.60	5.65	6.70	3.45	7.80	4.50	9.50	7.75	6.00	7.23	8.50	7.80
H. P. (II)	7.20	5.70	3.90	3.70	4.35	6.20	9.65	8.45	5.65	4.40	6.55	6.85
M. G.	10.90	8.70	12.40	9.50	9.90	8.40	21.80	12.00	17.50	17.00	18.40	10.50
J. Q.	6.70	3.70	5.55	3.10	5.35	4.00	9.00	4.25	7.90	4.10	6.70	4.70
Mean	7.09	5.36	6.14	4.38	6.07	4.97	10.76	7.61	7.83	7.00	8.47	6.40

variety of diseases, the response to positional changes is essentially the same as in the chronic pulmonary emphysema group. However, some interesting differences have to be pointed out. While in chronic pulmonary emphysema, a change in respiratory rate produces a significant drop of compliance in all positions, in this group it occurs only in the supine position. In addition, there is a significant difference in inspiratory resistance at different respiratory rates in the supine position, and in expiratory resistance in the supine and sitting positions. The results of the prone position in this group are not suitable for statistical analysis because of the small number of subjects.

3. *Head down position*

The five patients studied in the head down position (lying on the back at an angle of approximately 20 degrees) were selected from both patient groups. Compared with the supine position, the end expiratory pressure is found increased in all; with one exception, compliance is essentially equal and the resistance values are somewhat lower. However, only the difference in expiratory resistance is statistically significant. There is a significant decrease of compliance with increase in the respiratory rate in the head down position. Our results do not confirm the claim that the head down position is an important method for improving the ventilation in chronic pulmonary emphysema (7).

4. *Lateral position*

The six patients of this group were all expected to show some unilateral dysfunction. It is realized, however, that the latter could not be quan-

titatively evaluated without bronchospirometry. This does not invalidate the results which are interpreted on the assumption that intraesophageal pressure differences express the result of the intrapleural pressure differences on both sides. If the diseased side lies below, the end expiratory pressure is higher than if the healthy side lies below; the values for the supine position are usually somewhere between the two. In the four cases where simultaneous intrapleural and intraesophageal pressures were taken, the intrapleural compliance tended to be somewhat lower than the intraesophageal compliance if the patient was lying on the diseased lung and considerably higher if he was lying on the healthy side. However, the direction of change was the same for intrapleural and intraesophageal measurements. It must be kept in mind that during inspiration the hydrostatic pressure of a pleural effusion tends to fall because of the increase in thorax volume, thereby increasing the total pressure difference. This would lead to an apparent decrease of compliance, more marked in intrapleural measurements than in intraesophageal measurements, while mechanical resistance would increase. This is probably a minor factor in the supine position, where the differences in intrapleural and intraesophageal compliance are small. Both intrapleural and intraesophageal compliances are higher when the healthy lung is below as compared to the diseased lung below. There is no significant change in compliance with change in the respiratory rate. Mechanical resistance is higher during expiration than during inspiration in both lateral positions, but the differences are less marked when the healthy lung is below. Both inspiratory and expiratory resistance are lower in the healthy side down as compared to the diseased side down posi-

TABLE V

Mechanics of breathing: Results of six patients in supine, sitting and two lateral positions during slow and fast respiratory rates

	Respiratory rate								Tidal volume (*ml.*)							
	Supine		Sitting		D. Lat.*		H. Lat.†		Supine		Sitting		D. Lat.*		H. Lat.†	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
J. C.	20	100	6	100	22	65	24	60	560	245	592	270	530	385	490	440
W. F.	16	65	17	71	15	72	16	75	610	450	410	550	550	600	600	470
B. B.	20	50	30	70	22	55	25	50	520	380	490	330	500	385	500	430
H. T.	11	80	14	80	14	80	11	80	850	350	750	435	750	550	870	470
G. A.	15	83	26	100	23	78	23	86	670	330	650	350	630	390	670	330
A. A.	24	60			24	50	24	55	560	500			390	300	578	390
Mean	17.7	73	18.6	80.4	20	66.6	20.5	67.6	628	376	578	387	558	435	618	422

	Intraesophageal end expiratory pressure (*cm. H_2O*)								Compliance (*L./cm. H_2O*)							
	Supine		Sitting		D. Lat.*		H. Lat.†		Supine		Sitting		D. Lat.*		H. Lat.†	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
J. C.	+1.8	+0.9	+1.1	−2.0	+1.0	−0.5	−0.7	−1.0	0.143	0.162	0.161	0.161	0.115	0.136	0.205	0.220
W. F.	−0.8	+4.0	−9.0	−7.8	−1.3	−0.3	−3.5	−2.0	0.145	0.080	0.086	0.104	0.143	0.150	0.135	0.095
B. B.	−2.0	−6.2	−3.2	−8.5	−0.5	−3.0	−4.0	−6.0	0.045	0.028	0.060	0.031	0.060	0.031	0.056	0.034
H. T.	0	−1.2	−5.0	−5.0	−1.0	−0.5	−1.5	+0.5	0.112	0.112	0.200	0.210	0.120	0.127	0.160	0.160
G. A.	+1.5	+1.0	−1.5	−2.5	+4.5	+1.0	+2.5	−2.0	0.105	0.084	0.142	0.141	0.064	0.050	0.080	0.074
A. A.	+2.5	+3.0			+2.0	+0.5	+2.0	+0.5	0.087	0.072			0.050	0.038	0.090	0.072
Mean	+0.5	+0.5	−3.5	−5.2	+0.8	−0.5	−0.9	−2.2	0.106	0.090	0.130	0.130	0.092	0.089	0.121	0.109

	Mean mechanical resistance during inspiration (*cm. H_2O/L./sec.*)								Mean mechanical resistance during expiration (*cm. H_2O/L./sec.*)							
	Supine		Sitting		D. Lat.*		H. Lat.†		Supine		Sitting		D. Lat.*		H. Lat.†	
Patient	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR	SR	FR
J. C.	2.18	2.83	2.34	2.60	4.10	3.40	2.00	3.15	2.38	2.80	2.32	2.22	3.75	2.85	2.22	3.21
W. F.	3.18	2.41	2.20	3.14	2.30	2.48	1.68	5.65	6.80	4.46	3.45	3.27	5.30	3.93	6.05	8.65
B. B.	13.00	12.50	9.20	8.90	10.80	8.75	11.20	10.75	13.60	17.00	8.00	9.70	16.60	18.20	18.40	19.40
H. T.	1.50	2.40	1.65	1.00	1.00	1.65	2.36	1.70	2.50	3.00	2.70	1.30	2.00	2.35	2.72	2.27
G. A.	5.70	3.10	1.75	1.84	3.30	3.90	4.30	3.90	10.60	5.75	2.06	2.63	11.10	5.10	5.20	3.84
A. A.	4.75	5.25			10.30	6.85	5.60	4.30	8.80	5.50			10.00	8.90	4.95	3.00
Mean	5.05	4.75	3.43	3.50	5.30	4.48	4.53	4.81	7.45	6.42	3.70	3.80	8.12	6.88	6.60	6.72

* Lateral position: Lying on diseased side.
† Lateral position: Lying on healthy side.

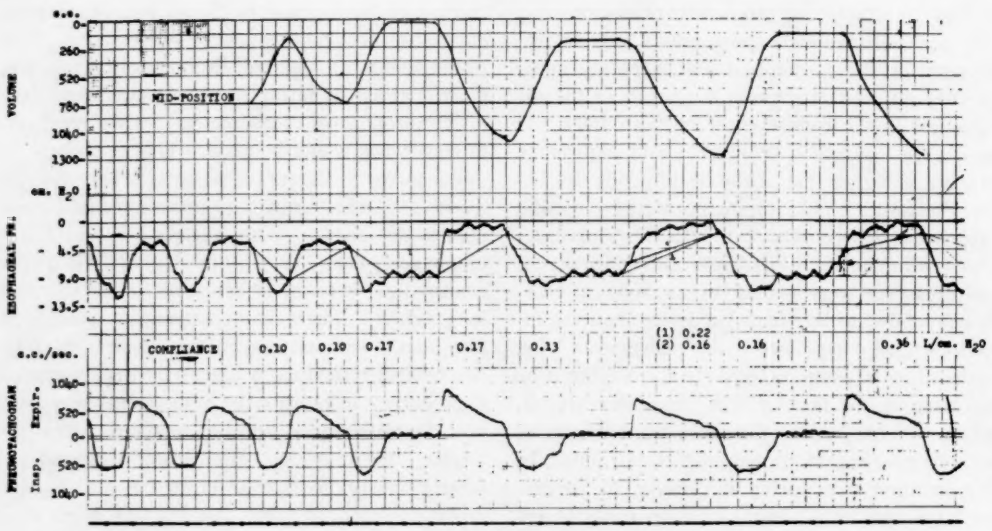


FIGURE 1

Change in compliance due to unequal distribution of air (sitting position with inspiratory pause and change of mid-position with respiratory rate). Change of end inspiratory pressure during the pause with transmission of heart beats. At a, glottis closure and change of intraesophageal pressure due to action of expiratory muscles with zero air flow.

tion. However, the scatter of the individual scores is quite large and the differences are not statistically significant as compared to the supine position.

DISCUSSION

The overall changes in the mechanics of breathing in the supine, sitting and prone positions for both groups of patients are essentially the same as for the normal group previously described.

There is, however, one essential difference between the two patient groups and the normal group. The normal subjects do not show any change in compliance or resistance with an increase in the respiratory rate. The chronic pulmonary emphysema group shows a marked drop of compliance with an increase in rate in all positions, while the resistance values remain essentially the same. The group with various cardiopulmonary diseases reveals a significant drop of compliance in the supine and prone positions, a significant drop of inspiratory resistance in the supine and of expiratory resistance in the sitting and supine positions. The dependence of compliance upon respiratory rate is shown in Figure 1 where compliance decreases with an increase of respiratory rate. This is usually explained on the basis of unequal ventilation (6, 8, 9). During increased respiratory rates, a smaller total volume change occurs per given pressure. In view of the fact that under these conditions a time element influences the compliance, the latter is no longer a pure expression of the elastic properties alone if measured by the described method. In an elastic system, the response to a force depends only on the magnitude of the force applied while in a viscous system, the response depends on the duration of the time during which the force is applied (10). According to Mount (11), part of the elastic element of the lungs is in series with a variable tissue deformation resistance which confers time dependency upon the elastic properties of the lung. The changes of compliance with different respiratory rates and different body positions, are shown together with the results of the seven-minute nitrogen washout in the supine (Figure 2) and in the supine and sitting (Figure 3) positions.

It will be seen that, in general, low compliance values go together with high intrapulmonary mixing indices. Inasmuch as most of these patients

tend to have an increased respiratory rate even under basal conditions, it is conceivable that the "basal" compliance which we obtain is already reduced and would be considerably higher if measured under static conditions. Furthermore, the nitrogen washout method tends to give too favorable a picture in hyperventilating subjects. These two factors could explain why this correlation is not perfect.

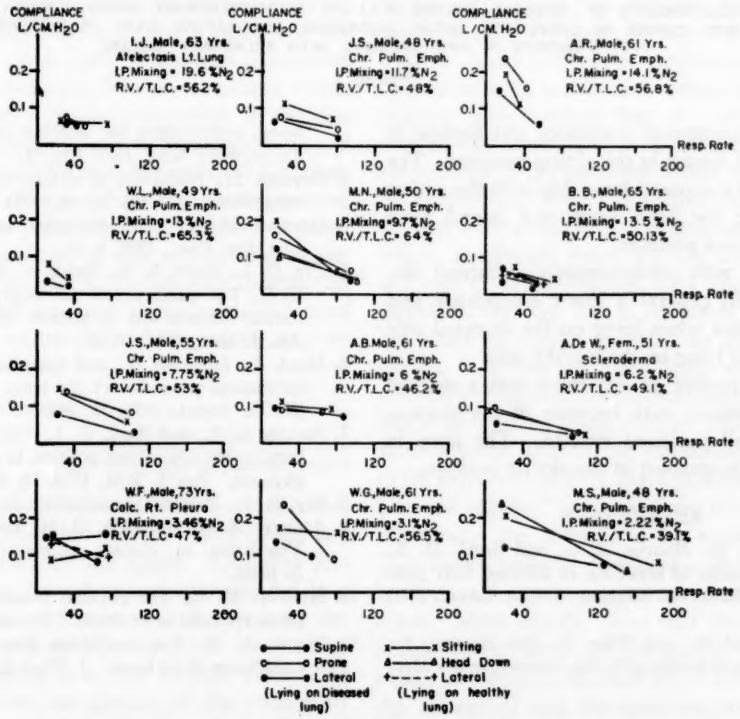
From our results it would seem that the supine, head down and lateral positions require more effort for the breathing process than the sitting and prone positions. They also are accompanied by poorer intrapulmonary mixing. Such changes become important in the patient with pulmonary insufficiency where minimal additional efforts may suffice to produce a severe and embarrassing picture (CO_2 narcosis, atelectasis, drug addiction, *etc.*). The striking relief of dyspnea which can be observed in many patients assuming the sitting position may also be explained by the improved mechanics of breathing noted in this position.

SUMMARY

1. Studies in the mechanics of breathing in a group of patients with chronic pulmonary emphysema and a group of patients with various cardiopulmonary diseases are reported. Compliance and mechanical resistance were measured during slow and fast respiratory rates in the supine, sitting, prone, head down and lateral positions.

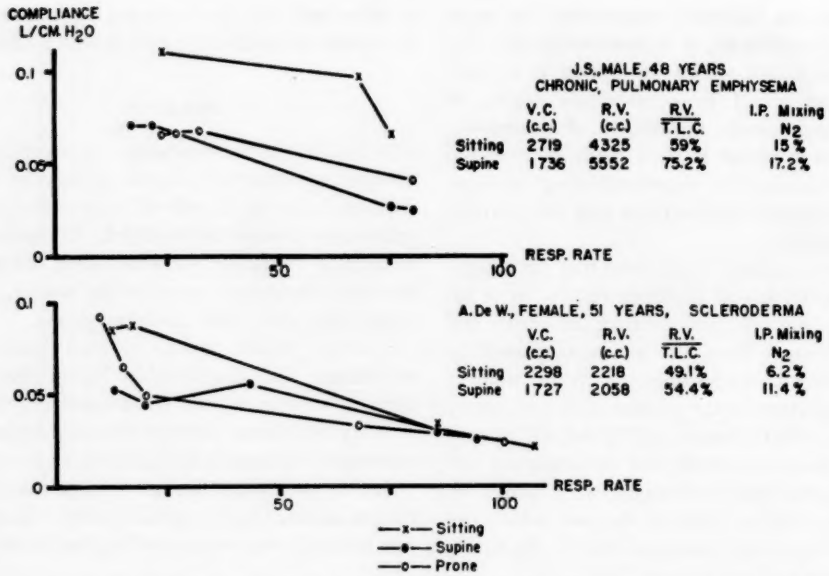
2. Both patient groups showed lower mean compliance and considerably higher mean mechanical resistance than the normal group. Expiratory resistance was considerably higher than inspiratory resistance in both patient groups.

3. The positional changes were in the same direction as noted in the normal group. Compliance was lowest in the supine and highest in the sitting


COMPLIANCE L/CM. H2O
I.J., Male, 63 Yrs. Atelectasis Lt. Lung I.P. Mixing = 19.6 %N2 R.V./T.L.C. = 56.2%
J.S., Male, 48 Yrs. Chr. Pulm. Emph. I.P. Mixing = 11.7 %N2 R.V./T.L.C. = 48%
A.R., Male, 61 Yrs. Chr. Pulm. Emph. I.P. Mixing = 14.1 %N2 R.V./T.L.C. = 56.8%
W.L., Male, 49 Yrs. Chr. Pulm. Emph. I.P. Mixing = 13 %N2 R.V./T.L.C. = 65.3%
M.N., Male, 50 Yrs. Chr. Pulm. Emph. I.P. Mixing = 9.7 %N2 R.V./T.L.C. = 64%
B.B., Male, 65 Yrs. Chr. Pulm. Emph. I.P. Mixing = 13.5 %N2 R.V./T.L.C. = 50.13%
J.S., Male, 55 Yrs. Chr. Pulm. Emph. I.P. Mixing = 7.75 %N2 R.V./T.L.C. = 53%
A.B. Male, 61 Yrs. Chr. Pulm. Emph. I.P. Mixing = 6 %N2 R.V./T.L.C. = 46.2%
A.De W., Fem., 51 Yrs. Scleroderma I.P. Mixing = 6.2 %N2 R.V./T.L.C. = 49.1%
W.F., Male, 73 Yrs. Calc. Rt. Pleura I.P. Mixing = 3.46 %N2 R.V./T.L.C. = 47%
W.G., Male, 61 Yrs. Chr. Pulm. Emph. I.P. Mixing = 3.1 %N2 R.V./T.L.C. = 56.5%
M.S., Male, 48 Yrs. Chr. Pulm. Emph. I.P. Mixing = 2.22 %N2 R.V./T.L.C. = 39.1%
Resp. Rate
0.2
0.1
40
120
200
Supine
Prone
Lateral (Lying on Diseased lung)
Sitting
Head Down
Lateral (Lying on healthy lung)

CORRELATION OF CHANGES IN COMPLIANCE WITH (1) RESPIRATORY RATE IN DIFFERENT BODY POSITIONS AND (2) INTRA-PULMONARY MIXING (%N_2) IN THE SITTING POSITION IN TWELVE PATIENTS WITH PULMONARY DISEASE

FIGURE 2


COMPLIANCE
L/CM. H_2O
0.1
0.05
0
50
100
RESP. RATE
J.S., MALE, 48 YEARS
CHRONIC, PULMONARY EMPHYSEMA
V.C. (c.c.) | R.V. (c.c.) | R.V./T.L.C. | I.P. Mixing N_2
Sitting 2719 | 4325 | 59% | 15%
Supine 1736 | 5552 | 75.2% | 17.2%
A. De W., FEMALE, 51 YEARS, SCLERODERMA
V.C. (c.c.) | R.V. (c.c.) | R.V./T.L.C. | I.P. Mixing N_2
Sitting 2298 | 2218 | 49.1% | 6.2%
Supine 1727 | 2058 | 54.4% | 11.4%
—x— Sitting
—●— Supine
—o— Prone

RELATIONSHIPS OF RESIDUAL VOLUME (R.V.) AND INTRA-PULMONARY MIXING (I.P.MIXING %N_2) WITH CHANGE IN COMPLIANCE DURING INCREASING RESPIRATORY RATE IN DIFFERENT BODY POSITIONS IN TWO PATIENTS WITH PULMONARY DISEASE.

FIGURE 3

position and mechanical resistance was highest in the supine and lowest in the sitting position. The prone position compared favorably with the sitting position while the head down and lateral were nearer the supine position.

4. Patients with predominantly unilateral disease showed in general a lower compliance and higher resistance when lying on the diseased side as compared to lying on the healthy side.

5. In both patient groups there was a marked drop of compliance with increase in the respiratory rate in all positions studied. The drop in compliance was smallest in the sitting position.

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A CONSTANT-CHANGE (SINGLE INJECTION) METHOD FOR THE ESTIMATION OF THE VOLUME OF DISTRIBUTION OF SUBSTANCES IN BODY FLUID COMPARTMENTS

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(Submitted for publication October 4, 1954; accepted April 24, 1956)

There are at least two problems in estimating the volume of a body fluid compartment. First, a substance must be found which is distributed in the volume to be measured, and second, a method must be made available which will measure the distribution of the substance. In the literature, substance and method are rarely, if ever, separated. Kruhoffer (1), for example, states that an ideal indicator of the extracellular fluid space should possess the following properties: "1) When sufficient time has elapsed for complete distribution the material must be found everywhere in the extracellular fluid and exclusively there and the concentrations of it must be uniform throughout the interstitial fluid and throughout plasma water, the ratio between the concentrations in the two compartments being a known fixed figure. 2) It must not be eliminable from the extracellular space. 3) It must not possess so high an osmotic pressure, that it causes a perceptible displacement of water from the intracellular to the extracellular space. 4) not to be toxic." When complete distribution has been attained for a non-electrolytic indicator of this kind, the extracellular fluid volume may be calculated as follows:

$$\text{Extracellular Volume} = \frac{\text{quantity injected}}{\text{concentration in plasma water}}$$

Since the *simple dilution method* is being used, the substance must be *uniformly distributed* and remain in the extracellular space. Kruhoffer further states that, for substances administered in a single dose, the replacement of the quantity injected by the quantity injected minus the quantity excreted in the urine is not without error—"In such a procedure, on account of the continuous excretion, an equilibrium between the concentrations of the indicator substances in plasma and interstitial fluid is never attained." To establish an equilibrium, Kruhoffer felt that two methods could be used: "1) After a large initial dose the substance might be administered by steady, continuous infusion to keep the plasma concentration constant until distribution was complete. 2) The organs of excretion (the kidneys) might be removed prior to administration." It is clear that equilibrium, as used by Kruhoffer for a non-electrolyte, means constant and equal concentrations of the indicator in both the interstitial fluid and plasma water. Whether or not such an equilibrium is obtained by either nephrectomy or a continuous infusion is difficult to prove by experimentation. It is evident that the *simple dilution method* has determined, to a certain extent, what the behavior of the substance should be in the body.

Guadino and Levitt (2) further defined an ideal substance for measuring extracellular fluid as one which distributed fairly rapidly and uniformly, and was eliminated slowly from the body. Furthermore, they felt that a substance such as inulin, which is rapidly excreted by glomerular filtration, is prevented from being uniformly distributed throughout the extracellular space after a single injection. These authors utilized the *constant-infusion method* for estimating the volume of the extracellular fluid, and again the method dictates the required behavior of the indicator. Fundamentally, the purpose of the constant infusion is to obtain uniform distribution of the substance between all compartments of the extracellular space. More exactly, since only the plasma concentration can be measured readily, the aim is to obtain the same concentration of the indicator in the interstitial and the intravascular spaces and to maintain this equal concentration over a long enough period so that it can be measured. Obviously, this cannot be done with a single injection

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because the indicator concentration is constantly changing in both compartments. However, this does not mean, as repeatedly reported (2–5), that a single injection of a substance cannot be used to measure the "extracellular volume." Most probably, a single injection cannot be used if the calculation for the volume of distribution of the indicator is made on the basis of the constant-infusion method. The method which is used to determine the distribution of the substance must necessarily take into account the behavior of the substance after it has been introduced into the body. With the constant-infusion method, equilibrium is indicated by a constancy of the plasma concentration of the indicator, and the error involved in assuming uniform distribution of the indicator may well be insignificant.

Another commonly used single injection method for estimating the volume of distribution of substances is the *extrapolation method,* as used, for example, by Cardozo and Edelman (6). Serum concentrations are plotted semi-logarithmically against time and the "equilibrium" concentration is obtained by extrapolation to the time of commencement of infusion (zero time). Theoretically, the method can only be used with substances which have a constant clearance from the time of the injection. Newman, Bordley, and Winternitz (7) assumed constant clearance of the substance in deriving the equation which relates the volume of distribution of a non-metabolized substance to the renal clearance and the time decrement of the natural logarithm of the plasma concentration. Again, the method severely restricts the permissible behavior of the substance.

The purpose of this communication is to introduce a single injection method, given the name of the constant-change method, for calculating the apparent distribution of substances in an intact animal. The method is independent of the amount of indicator injected, and from the theoretical point of view, does not require that the substance be uniformly distributed in the fluid compartment which is being measured and, thus treats the plasma and interstitial concentrations of the indicator as being unequal and constantly changing. The method has been compared directly with two single injection methods, the simple dilution (with excretion) and extrapolation methods, and with the constant-infusion method. Inulin was used as the indicator in man, and simultaneously injected mannitol and sucrose was used in dogs. The method has been found to be superior to the other single injection methods, and at least equal to the constant-infusion method. An attempt has been made to define steady state conditions between plasma and interstitial fluids after a single injection of an indicator.

THEORETICAL CONSIDERATIONS

The derivation of the general equation used for the estimation of one fluid compartment will be considered first. Then, similar equations for dealing with two or more compartments will be developed.

1. *One compartment:* If a given amount of a substance, A, is injected into a fluid volume, V, and U is the loss of the substance from the volume (Figure 1a), then the amount of substance injected must be equal to the amount of substance in the volume plus that which has been lost. Thus, if no substance is created or destroyed, then

$$A = CV + U \tag{1}$$

where C equals the average concentration of the substance in volume V. If now one differentiates with respect to time,

$$0 = V\frac{dC}{dt} + \frac{dU}{dt} \tag{2}$$

or

$$V = -\frac{dU/dt}{dC/dt} \tag{3}$$

or

$$\text{Volume} = \frac{\text{Instantaneous rate of change of U with time}}{\text{Instantaneous rate of change of C with time}}$$

2. *Two compartments:* The above volume, V, is now divided into two compartments, V_1 and V_2, which are separated by a permeable membrane so that V_2 is about three times larger than V_1 (Figure 1b). The substance A is injected into volume V_1 and distributes into volume V_2. At any time after the injection of the indicator, the continuity equation becomes

$$A = C_1V_1 + C_2V_2 + U \tag{4}$$

where

- A = amount of substance injected into compartment V_1;
- C_1 = average concentration of the substance in compartment V_1;
- C_2 = average concentration of the substance in compartment V_2;
- U = amount of substance lost from the volume.

An expression must be obtained for the total volume, $V_1 + V_2$. So to equation (4) add and subtract the quantity C_1V_2,

$$A = C_1V_1 + C_2V_2 + U + C_1V_2 - C_1V_2$$

and regroup the terms, so that

$$A = C_1(V_1 + V_2) + V_2(C_2 - C_1) + U$$

Now, differentiating with respect to time, one obtains,

$$0 = (V_1 + V_2)\frac{dC_1}{dt} + V_2\frac{d}{dt}(C_2 - C_1) + \frac{dU}{dt}$$

or

$$V_1 + V_2 = -\frac{V_2\frac{d}{dt}(C_2 - C_1) + \frac{dU}{dt}}{dC_1/dt} \qquad (5)$$

If C_2, dU/dt, and dC_1/dt cannot be measured, certain simplifying assumptions must be made in order to obtain a useful equation. The quantity $(C_2 - C_1)$ may be assumed to remain constant, then the term $V_2\frac{d}{dt}(C_2 - C_1)$ becomes zero. In other words, if the difference in the concentrations of the substance in volumes V_1 and V_2 remains constant, the term $V_2\frac{d}{dt}(C_2 - C_1)$ becomes zero because the derivative of a constant is zero, and the expression becomes

$$V_1 + V_2 = -\frac{dU/dt}{dC_1/dt} \qquad (6)$$

or

$$\text{Total Volume} = \frac{\text{Instantaneous rate of change of U with time}}{\text{Instantaneous rate of change of } C_1 \text{ with time}}$$

It should be noted that, in assuming that $C_2 - C_1$ remains constant, the constant may vary from zero to infinity. If the constant is zero, then $C_2 = C_1$, and this would indicate that uniform distribution of the indicator is assumed. However, the most general assumption would be that $C_2 - C_1$ equals something other than zero.

3. *Three compartments:* If the volume were divided into three compartments, V_1, V_2, and V_3, by permeable membranes the equation would become

$$A = C_1V_1 + C_2V_2 + C_3V_3 + U$$

If one now adds and subtracts C_1V_3 as well as C_1V_2 and differentiates, then

$$0 = (V_1 + V_2 + V_3)\frac{dC_1}{dt} + V_2\frac{d}{dt}(C_2 - C_1) + V_3\frac{d}{dt}(C_3 - C_1) + \frac{dU}{dt}$$

Now, if the difference in the concentration of the substance in volumes V_3 and V_1 is constant, then $(C_3 - C_1)$ becomes zero. And as above if $(C_2 - C_1)$ again becomes zero, then

$$V_1 + V_2 + V_3 = -\frac{dU/dt}{dC_1/dt} \qquad (7)$$

or

$$\text{Total Volume} = \frac{\text{Instantaneous rate of change of U with time}}{\text{Instantaneous rate of change of } C_1 \text{ with time}}$$

4. *Practical application of the theory:* Since one cannot measure the instantaneous rates of change of U and C with time, Equations *3*, *6*, and *7* are not in a useful form. One must make further simplifying assumptions, without deviating significantly from the theory, in the hope of obtaining a practical equation.

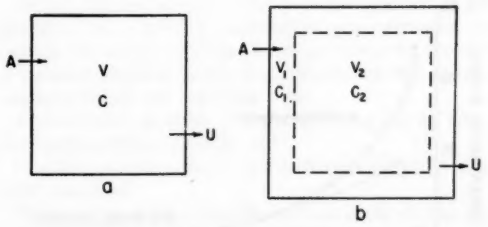

FIG. 1. a) ONE COMPARTMENT

A equals the amount of substance injected into volume V. U is the loss of substance from V, and the average concentration of the substance is C.

b) TWO COMPARTMENTS, V_1 AND V_2 SEPARATED BY A PERMEABLE MEMBRANE

The average concentrations of the substance in each compartment is C_1 and C_2.

Let us consider inulin as an indicator which is distributed in two phases of the extracellular fluid. Referring to Figure 1b, the following is defined:

A = amount of inulin injected into volume V_1 in mg.
V_2 = interstitial volume in cc.
V_1 = intravascular volume in cc.
C_1 = average plasma concentration of inulin in mg. per cent.
C_2 = average interstitial concentration of inulin in mg. per cent.
U = urinary excretion of inulin in mg.
$V_{EC} = V_1 + V_2$ = volume of distribution of inulin.

After a single intravenous injection of inulin, the plasma inulin concentration versus time curve has the form shown in Figure 2, and the total urinary inulin excretion versus time curve has the form shown in Figure 3. Moreover, by measuring the plasma volume and the "extracellular volume" (volume of distribution of inulin) the virtual interstitial concentration of inulin may be calculated (4), and related to the plasma inulin concentration (Figure 2). It is important to note that soon after the plasma and the interstitial inulin concentrations are equal (where the curves cross at P) the curves are close together and appear to become parallel, with the interstitial inulin concentration at a higher value than the plasma inulin concentration. Now, referring to Equation *5*, the error involved in assuming that the term $(C_2 - C_1)$ is constant may not be significant. Most likely, C_2 is equal to C_1 at some time P, then C_2 is greater than C_1 and at infinity both C_1 and C_2 are equal to zero. For relatively short intervals of time, the two curves may be assumed to be parallel. Equation *6* may then be considered as an expression for the volume of distribution of inulin. This equation is valid only over the range of plasma inulin concentrations where the difference between interstitial and plasma inulin concentrations remains constant, or very nearly constant. This limitation in the use of the equation defines an *equilibrium condition* which should exist, or be approached, after a single injection of an indicator is given. As in other methods, the

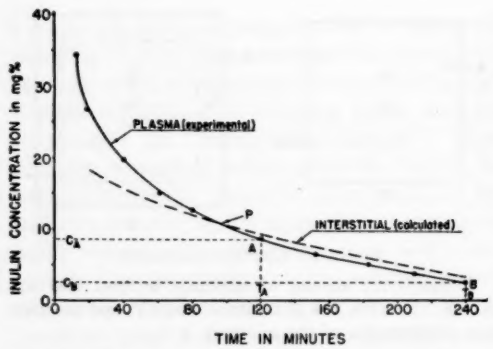

FIG. 2. RELATIONSHIP OF THE CALCULATED INTERSTITIAL INULIN CONCENTRATION TO THE PLASMA INULIN CONCENTRATION, FOLLOWING A SINGLE INTRAVENOUS INJECTION OF THIS SUBSTANCE AT TIME ZERO

acceptable behavior of the substance is limited by the method.

If during this period, where the difference between plasma and interstitial inulin concentrations is assumed to remain constant, the plasma concentration and urinary excretion of inulin versus time curves approach a straight line, then there will be a small but probably insignificant error in assuming linear dependence of plasma concentration and urinary excretion with time. By making this assumption, the change of plasma inulin concentration over a finite period of time may be measured and substituted in Equation *6* for the instantaneous rate of change of the plasma concentration with time. The change in urinary excretion of inulin over the same finite period of time can also be measured and substituted in the equation. For example, the plasma concentration of inulin is determined at 120 and 240 minutes after a single injection (A and B, Figure 2), and during this time the excreted inulin is measured (A to B, Figure 3). The apparent volume of distribution of inulin is then calculated as follows:

$$\text{V. of D. of Inulin} = -\frac{U_B - U_A/T_B - T_A}{C_B - C_A/T_B - T_A}$$

Since measurements are made over the same time interval, the equation simply becomes:

$$\text{Volume of Distribution of Indicator} = \frac{U_B - U_A}{C_A - C_B} \quad (8)$$

or

$$\text{V. of D. of Indicator} = \frac{\text{Urinary excretion of indicator during time A to B}}{\text{Change in plasma concentration of the substance during the same time interval}}$$

In summary, the measurements should be made during the period where there is a constant, or nearly constant, difference between interstitial and plasma inulin concentration. Moreover, in order to follow the theory as closely as possible, that portion of the curve which most closely approximates a straight line should be used. Any amount of inulin is injected intravenously at time zero. At time A a blood sample is drawn and all urine up to this time is discarded. Another blood sample is drawn at time B and all urine is collected from time A to B. After the appropriate corrections are made, the volume of distribution of the substance is calculated by dividing the urinary excretion of inulin during the time interval from A to B by the change in plasma inulin concentration during the same period. It is not necessary to know how much inulin was used because the calculation of the volume of distribution of the substance is independent of the amount of inulin injected.

Sample Calculation (see Figures 2 and 3).

After a single injection of about 4 gm. of inulin, the plasma water concentration was found to be 8.5 mg. per cent at 120 minutes and 2.5 mg. per cent at 240 minutes after injection. During this interval, 890 mg. of inulin were excreted in the urine:

$$\text{V. of D. of Inulin} = \frac{890}{8.5 - 2.5} \times 100 = 14.8 \text{ liters}$$

N.B. In the constant-change method there is no need for correcting the plasma inulin concentration for the plasma inulinoid blank, because only the *difference* in plasma concentration is required for calculating the volume of distribution and not the actual values.

METHODS

Fifteen experiments on 6 normal healthy male physicians and 8 experiments on 4 adult mongrel female dogs comprise the basis of this report.

Inulin: Inulin was used as an indicator of the body fluid compartment measured in man. The subjects fasted from midnight the night preceding the test until the end of the experiment. On arising in the morning of the test, they emptied their bladders and drank two to three glasses of water. Samples of blood and urine were taken for the determinations of the concentration of inulinoid material. One intravenous injection of inulin was given, with the dose accurately measured in only one subject. Most subjects received approximately 50 cc. of 10 per cent inulin. Blood samples were collected from

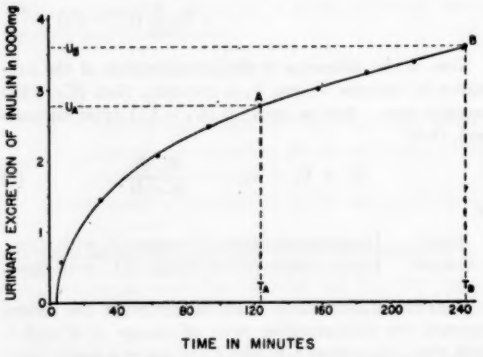

FIG. 3. TOTAL URINARY EXCRETION OF INULIN FOLLOWING A SINGLE INTRAVENOUS INJECTION OF THIS SUBSTANCE AT TIME ZERO

an antecubital vein at 150, 180, 240, and 270 minutes after the injection. The urinary bladder was emptied by voiding, spontaneously, three to five minutes after each blood sample was drawn. To maintain an adequate urine flow, each individual drank 150 cc. of water every 30 to 60 minutes during the course of the experiment. The subjects were lying down throughout the test except for a few moments required to collect a urine sample. In a preliminary experiment, blood and urine samples were taken continuously every 20 to 30 minutes after a single injection of inulin, and the plasma volume was also determined, simultaneously, using T-1824 dye.

Mannitol and sucrose: The volumes of distribution of mannitol and sucrose were studied in dogs, anesthetized with sodium pentobarbital. The constant-infusion method, as described by Deane (8), was used in each of 4 dogs. Following a priming dose (1.5 gm. of each substance), a constant infusion of mannitol and sucrose (300 mg. per cent of each) in normal saline was given at the rate of 5 cc. per minute for a period of 2 hours. Three blood samples, fifteen minutes apart, were then taken to check the constancy of the plasma concentration of the indicator. After washing out the bladder, a normal saline infusion was given at a rate of 2 cc. per minute, and the urine was collected for at least 6 hours. Blood samples were withdrawn from the jugular vein and an indwelling catheter was used to collect the urine.

The same 4 dogs were used to study the three single injection methods. The dogs were anesthetized and catheterized, as above. An intravenous infusion of normal saline was given at the rate of 5 cc. per minute. After the urine flow became adequate (1 to 3 cc. per minute), urine and blood blank samples were taken, and a single injection of 5 gm. of sucrose and 5 gm. of mannitol was given intravenously within 10 seconds. The saline infusion was maintained at 5 cc. per minute throughout the experiment. Blood samples were drawn at 120, 150, 180, and 210 minutes after the injection. Urine samples were collected 2 minutes after each blood sample, and the bladder was washed out once with saline and air at the end of each collection period.

In all experiments, the subject was weighed at the end of the test. A careful record of fluid intake and output was kept in the dog experiments.

Analytical methods: Inulin and sucrose were determined by Schreiner's resorcinol technique (9), mannitol by the method of Corcoran and Page (10) and plasma volume was estimated by dilution of T-1824 dye using the procedure described by Chinard (11). Inulin determinations were made in duplicate, and mannitol and sucrose determinations in triplicate. In the animal experiments, plasma concentrations were converted to plasma water concentrations by dividing by 0.94.

CALCULATIONS

Constant-infusion method: The volumes of distribution of sucrose and mannitol, obtained by using the constant-infusion method, were calculated by using the formula given by Deane (8).

Simple dilution method: After a single injection of an indicator, the volume of distribution was calculated as equal to the amount of material injected minus the amount of material excreted in the urine divided by the plasma concentration at any particular time.

Extrapolation method: The method described by Cardozo and Edelman (6) was used.

Constant-change method: The method described in this paper was used.

Wherever possible a comparison was made of the results obtained by each of these methods.

RESULTS

1. *The volume of distribution of inulin in man*

Since Equation *8* indicates that the calculation of the volume of distribution of a substance is independent of the amount of indicator given, a series of experiments were performed on the same individual using varying amounts of inulin as the indicator. Table I summarizes the results of these experiments. The subject received from 3008 to 5000 mg. of inulin on five different days. The volume of distribution of inulin by the constant-change calculation was found to be 16.2, 16.0, 16.2, 16.2,[2] and 15.6[2] per cent of body weight. The mean value was 16.0 per cent and the standard deviation ± 0.25 per cent of body weight.

[2] Mean values.

The same data were used to calculate the volume of distribution by the simple dilution and extrapolation methods. The results of these calculations are also given in Table I. In all experiments the value for the volume of distribution of inulin obtained by either the simple dilution or extrapolation methods *was higher than the figure obtained* by the constant-change method. The simple dilution method indicates that the volume of distribution of inulin increases with time (from 30.3 to 59.2 per cent on 3-15-54). In this series of experiments a fair comparison has not been made with the extrapolation method, since only two values are used for the extrapolation in most experiments.

Table II summarizes the results of 10 experiments on 5 different individuals who received a single intravenous injection of inulin (about 5 gm.) on two occasions. The volume of distribution has been calculated for three time intervals (150 to 240, 150 to 270, and 180 to 270 minutes after the injection). The mean value of the three periods is considered to be the best measure of the

TABLE I

The volume of distribution of inulin in the same individual using different amounts of inulin with a comparison of three single injection methods

Subject	Date	Body weight	Inulin injected	Time after injection of inulin	Cumulative inulin in urine	Plasma inulin conc.	Inulin volume of distribution: Simple dilution method		Extrapolation method		Constant-change method*: Time interval†		
		Kg.	*mg.*	*min.*	*mg.*	*mg. %*	*Lit.*	*% BW*	*Lit.*	*% BW*	*min.*	*Lit.*	*% BW*
P. P. P.	2/22/54	73.4	3,008	150	2,110	4.79	18.7	25.5			150–220	11.9	16.2
				220	2,350	2.77	23.8	32.4	19.8	27.0			
	3/5/54	73.1	3,050	150	2,080	5.00	19.4	26.5			150–240	11.7	16.0
				240	2,360	2.60	26.5	36.2	20.7	28.2			
	3/12/54	73.2	3,150	150	2,130	5.10	20.0	27.3			150–240	11.9	16.2
				240	2,403	2.80	26.6	36.3	22.9	31.3			
	3/15/54	73.0	4,226	150	2,810	6.40	22.1	30.3			150–240	11.6	15.9
				180	2,959	4.70	26.9	36.9			150–270	11.1	15.2
				240	3,194	3.10	33.3	45.6					
				270	3,276	2.20	43.2	59.2	19.0	26.0	180–270	12.7	17.4
											Mean	11.8	16.2
	3/17/54	72.8	about 5,000	150	3,850	8.90					150–240	11.3	15.6
				180	4,075	7.20					150–270	11.7	16.1
				240	4,393	4.10					180–270	11.1	15.2
				270	4,507	3.30							
											Mean	11.4	15.6
							Mean of 5 experiments					11.7	16.04
							Range of 5 experiments					11.4 to 11.9	15.6 to 16.2
							S.D. of 5 experiments						±0.25

* Equation *8* was used.
† Time interval after single injection of inulin used for calculating the volume of distribution of inulin.

volume of distribution of inulin. A series of three experiments (R. H. F., J. E. P., and H. V. B.) showed a variation in the volume of distribution of inulin of less than one per cent of body weight. In one experiment (R.D.Q.) the variation was large (2.1 per cent), and the values obtained for the (150 to 240) and (150 to 270) time intervals differed by more than 15 per cent both on 3-30-54 and 4-17-54.

The mean value of the volume of distribution of inulin in the six individuals was found to be 18.7 per cent of body weight with a standard deviation of ± 1.57 per cent.

2. *The volume of distribution of sucrose and mannitol in dog*

Since Swan, Madisso, and Pitts (12) found in nephrectomized dogs that the volume of distribution of sucrose averaged 91 per cent (84 to 99 per cent) of the mannitol volume, a series of experiments were designed to determine whether or not a similar result would be obtained on intact animals. Moreover, the methods used to calculate the volume of distribution of a substance could be compared.

Table III summarizes the results of four experiments on four dogs, which received a single injection of 5 gm. of sucrose and 5 gm. of mannitol. Observations of the plasma level of the indicators were made at 120, 150, 180, and 210 minutes after the injection. The amount of inulin excreted in the urine was measured at the end of each period. It is clear from Table III that the simple dilution method never gives a relatively constant value for the volume of distribution of a substance from 120 to 210 minutes after the single injection. The calculated volume of distribution of sucrose may vary as much as 162 per cent of body weight (Exp. 13, 54.1 to 216 per cent), and the calculated mannitol distribution may increase

TABLE II

The volume of distribution of inulin in different individuals on two occasions using the constant-change (single injection) method

Subject	First determination					Second determination					Differences		Average inulin volume of distribution	
	Date	Body weight	Period after injection	Inulin volume of distribution		Date	Body weight	Period after injection	Inulin volume of distribution					
		Kg.	*min.*	*Liters*	*% BW*		*Kg.*	*min.*	*Liters*	*% BW*	*Liters*	*% BW*	*Liters*	*% BW*
R. H. F.	3/27/54	90.5	150–240	16.5	18.3	4/5/54	89.6	150–240	14.8	16.7				
			150–270	15.5	17.2			150–270	15.3	17.0				
			180–270	15.5	17.2			180–270	17.5	19.8				
			Mean	15.8	17.5			Mean	15.9	17.8	+0.1	+0.3	15.85	17.6
J. E. P.	4/5/54	70.4	150–240	13.2	18.7	4/9/54	69.3	150–240	12.8	18.4				
			150–270	13.3	18.9			150–270	13.8	19.9				
			180–270	16.7	23.7			180–270	14.8	21.3				
			Mean	14.4	20.4			Mean	13.8	19.5	−0.6	−0.9	14.10	19.9
R. L.	4/1/54	64.9	150–240	13.8	21.4	4/21/54	64.5	150–240	12.9	20.0				
			150–270	12.3	19.0			150–270	11.5	17.9				
			180–270	13.9	21.4			180–270	12.8	19.8				
			Mean	13.3	20.6			Mean	12.7	19.3	−0.6	−1.3	13.0	19.9
H. V. B.	4/6/54	83.4	150–240	14.9	17.9	5/12/54	83.6	150–240	15.0	18.0				
			150–270	15.2	18.2			150–270	16.2	19.4				
			180–270	16.8	20.2			180–270	17.2	20.6				
			Mean	15.6	18.8			Mean	16.1	19.3	+0.5	+0.5	15.85	19.0
R. D. Q.	3/30/54	80.4	150–240	16.5	20.6	4/17/54	80.6	150–240	19.1	23.6				
			150–270	14.2	17.6			150–270	15.8	19.7				
			180–270	14.2	17.6			180–270	15.2	18.8				
			Mean	14.9	18.6			Mean	16.7	20.7	+1.8	+2.1	15.8	19.6

Mean value from table I	16.0
Mean of 6 individuals	18.7
Range of 6 individuals	16.0–20.0
S.D. of 6 individuals	±1.57

TABLE III

The volumes of distribution of sucrose and mannitol in dog obtained by using three different single injection methods

Exp. No. Weight	Time after injection of sucrose and mannitol	Cumulative sucrose in urine	Plasma sucrose conc.	Sucrose volume of distribution: Simple dilution method		Sucrose volume of distribution: Extrapolation method		Sucrose volume of distribution: Constant-change method: Time interval			Cumulative mannitol in urine	Plasma mannitol conc.	Mannitol volume of distribution: Simple dilution method		Mannitol volume of distribution: Extrapolation method		Mannitol volume of distribution: Constant-change method: Time interval		
Kg.	*min.*	*mg.*	*mg. %*	*cc.*	*% BW*	*cc.*	*% BW*	*min.*	*cc.*	*% BW*	*mg.*	*mg. %*	*cc.*	*% BW*	*cc.*	*% BW*	*min.*	*cc.*	*% BW*
11	120	3,520	22.0	6,720	48.0						3,570	18.00	7,950	56.0					
14.0	150	3,754	14.8	8,420	60.0			120–150	3,060	21.8	3,833	12.90	9,030	64.0			120–150	4,120	29.4
	180	3,929	9.12	11,700	83.5			150–180	2,900	20.7	4,012	7.40	13,300	95.0			150–180	2,950	21.0
	210	4,050	5.60	17,000	121.0	3,520	25.1	180–210	3,230	23.0	4,135	3.90	22,200	158.0	7,530	53.8	180–210	3,310	23.6
Mean									3,060	21.8								3,460	24.7
12	120	3,940	18.50	5,730	35.1						3,692	20.80	6,280	38.5					
16.3	150	4,185	12.00	6,800	41.6			120–150	3,540	21.7	3,972	14.40	7,140	43.8			120–150	4,110	25.2
	180	4,336	7.13	9,310	57.1			150–180	2,920	17.9	4,148	10.87	7,850	48.1			150–180	4,680	28.7
	210	4,435	4.60	12,300	75.5	4,620	28.3	180–210	3,660	22.4	4,257	8.10	9,170	56.2	8,260	50.6	180–210	3,700	22.7
Mean									3,370	20.7								4,160	25.5
13	120	3,420	20.1	7,850	54.1						3,520	24.2	6,100	42.0					
14.5	150	3,698	13.5	9,650	66.5			120–150	3,960	27.2	3,817	17.0	6,950	47.9			120–150	3,880	26.8
	180	3,874	7.07	16,000	110.0			150–180	2,580	17.8	4,020	10.6	9,240	63.6			150–180	2,990	20.6
	210	3,991	3.55	31,400	216.0	5,000	34.4	180–210	3,130	21.6	4,156	7.27	11,600	80.0	4,550	31.4	180–210	3,840	26.4
Mean									3,220	22.2								3,570	24.6
14*	120	3,405	23.7	4,610	31.6						3,390	24.3	4,560	31.5					
14.6	150	3,690	16.3	4,960	33.9			120–150	3,620	24.8	3,710	19.0	4,160	28.4			120–150	5,670	38.8
	180	3,897	10.9	5,430	37.2			150–180	3,610	24.7	3,962	12.5	4,310	29.5			150–180	3,630	24.9
	210	4,051	7.75	5,800	39.7	5,000	34.2	180–210	4,600	31.5	4,152	9.25	3,760	25.8	6,920	47.5	180–210	5,500	37.6
Mean									3,940	27.0								4,930	33.7

* Dog D (Exp. 14) had a post-operative throat infection following resection of the vocal cords, between 10–5–55 and 11–2–55. Loss in weight amounts to 15.6 per cent of original weight and is the result of poor nutrition.

by 102 per cent of body weight (Exp. 11, 56.0 to 158 per cent).

The extrapolation method indicates that the volume of distribution of sucrose may vary by 9.3 per cent (25.1 to 34.4 per cent) in different animals, whereas, the mannitol distribution may vary by 22.4 per cent of body weight (31.4 to 53.8 per cent). However, all the values obtained by the extrapolation method may not be correct. The plotted values of the logarithm of the plasma concentration against time must demonstrate linearity within the limits of error of the experiment or extrapolation is invalid (6). This requirement was not adequately fulfilled in some experiments, and the variation in the mannitol distribution may indicate that the extrapolation was unacceptable. Regardless of the source of error, the fact remains that the entire experiment must be repeated in order to obtain a single value of the volume of distribution of a substance by the extrapolation method.

The volumes of distribution of both sucrose and mannitol remained relatively constant during the 120 to 210-minute period of the experiment. In experiment 11, the distribution of sucrose increased from 3060 cc. (120 to 150 min.) to 3230 cc. (180 to 210 min), and from 120 to 210 minutes the animal retained 224 cc. of the infusion. This small change in body fluid was not reflected in the volume of distribution of mannitol. Until further experimentation proves otherwise, the mean value of the three periods is considered to be the best measure of the volume of distribution of the indicator in these experiments. In experiments 11, 12, and 13 sucrose was distributed in a body fluid volume which represented, respectively, 21.8, 20.7 and 22.2 per cent of body weight. In these same experiments, the volume of distribution of mannitol was found to be 24.7, 25.5, and 24.6 per cent of body weight.

At an earlier date, the constant-infusion method was used to determine, simultaneously, the volume of distribution of sucrose and mannitol in the four dogs of the above experiments. A comparison of the volumes of distribution of sucrose and mannitol, obtained by the constant-infusion and constant-change (single injection) methods, is presented in Table IV. In all experiments the constant-infusion method gave smaller values for the distribution of the indicators than the constant-change method. The average value for the volume of distribution of sucrose was 19.4 per cent body weight by the constant-infusion method, and 21.6 per cent by the single injection method. Mannitol was found to be distributed in a larger body fluid volume than sucrose by both methods. The

TABLE IV

The volumes of distribution of sucrose and mannitol in dogs obtained by the constant-infusion and constant-change (single injection) methods

	Dog A		Dog B		Dog C		Dog D		Mean values	
	Constant infusion method	Single injection method	Constant infusion method	Single injection method	Constant infusion method	Single injection method	Constant infusion method	Single injection method	Constant infusion method	Single injection method
Exp. No.	6	11	9	12	7	13	4	14*		
Date	10-10-55	10-24-55	10-17-55	10-26-55	10-12-55	10-31-55	10-5-55	11-2-55		
Weight—Kg.	15.3	14.0	16.35	16.3	15.1	14.5	17.3	14.6*		
Sucrose volume of distribution										
cc.	2,958	3,060	3,085	3,370	2,926	3,220	3,450	3,940		
% BW	19.3		18.9		19.4		20.0		19.4	
% BW		21.8		20.7		22.2		27.0†		21.6
Mannitol volume of distribution										
cc.	3,440	3,460	3,624	4,160	3,560	3,570	4,310	4,930		
% BW	22.5		22.2		23.5		24.9		23.3	
% BW		24.7		25.5		24.6		33.7†		24.9
Sucrose/mannitol										
%	85.8		85.1		82.6		80.3		83.4	
%		88.2		81.2		90.2		80.2		84.9

* See note, Table III.
† Not included in mean.

distribution of mannitol was 23.3 per cent body weight by the infusion method, and 24.9 per cent by the single injection method. The volume of distribution of sucrose, as a per cent of mannitol distribution, was 85.8, 85.1, 82.6, and 80.3 (mean 83.4) by the constant-infusion method, and 88.2, 81.2, 90.2, and 80.2 (mean 84.9) by the method of constant change. The variation is less by the infusion method (80.3 to 85.8) than by the single injection method (80.2 to 90.2).

The results obtained in experiment 14 (Table III and IV) are of particular interest in that the dog underwent a period of starvation after the constant-infusion and before the constant-change experiments were performed. The dog lost 15.6 per cent body weight (17.3 to 14.6 kg.). Both the sucrose and mannitol volumes of distribution were significantly greater than in the "normal" dogs of experiments 11, 12, and 13 (27.0 as compared to 21.8, 20.7, and 22.2 per cent for sucrose and 33.7 as compared to 24.7, 25.5, and 24.6 per cent for mannitol).

DISCUSSION

1. Comparison with other methods

It is difficult to compare the various methods used for the estimation of the volume of distribution of a substance, because the limitations of each method must be carefully considered. Furthermore, the "ideal" properties and acceptable behavior of the indicator may vary with each method. Moreover, after the substance has been introduced into the body, the "equilibrium conditions" for one method may not satisfy the equilibrium, or steady state, requirements of another method. Nevertheless, an attempt will be made to compare the constant-change method with the simple dilution, extrapolation, and constant-infusion methods.

The data presented in Tables II and III indicate that the volume of distribution of the indicator, calculated on the basis of the simple dilution method, does not remain constant or even relatively constant, between 120 and 270 minutes after the start of the experiment. It may be that the proper time intervals were not used, or the plasma concentrations of the indicator may have been too low (mostly below 20 mg. per cent) for accurate analysis. Elkinton (13) found that, in a majority of experiments on man, the volume of distribution of mannitol remained unchanged between 1 and 3 hours, when the plasma mannitol concentration was kept relatively high (about 20 to 80 mg. per cent). He felt that, after 3 hours, the analytical error, in determining the plasma level of mannitol, was too great to draw any conclusions. Using Elkinton's data (13), the volume of distribution of mannitol was calculated by the constant-change method and compared with the values obtained by the simple dilution method (Table V). Elkinton felt that equilibrium was attained between 30 and 60 minutes after a single injection of mannitol had been given. Equilibrium was indicated when the plot of the logarithm of the plasma concentration of mannitol against time became a straight line, and also, by a constant value for the volume of distribution between two separate points. Table V shows that the volume of distribution of mannitol, during the 120 to 180-minute interval, by the constant-change method remains very constant in one individual on three occasions (RE–21.0, 20.7, and 20.7 per cent), and fairly constant in another individual on two occasions (LG–22.1 and 21.4 per cent). Moreover, "equilibrium," as regards the constant-change method, probably was not attained in subject RE until 120 minutes, since the values for the distribution of mannitol are low and inconstant before this time. Here is an example of the meaning of equilibrium changing with the method which is used to calculate the volume of distribution of the substance. Furthermore, Kruhoffer's definition of equilibrium, constant and equal concentrations of the indicator in both the interstitial fluid and plasma water, differs from the above and meets the requirements of calculating the distribution of a substance by another method.

The amount of material injected into the body must be measured accurately in using the simple dilution method, but may be ignored in the constant-change method (Equation *8* and Table III). In the simple dilution method, as well as in the extrapolation and constant-infusion methods, the indicator must be completely excreted or accounted for before the distribution of a substance can be determined following a second injection. There is no such limitation in the constant-change method.

It would not be fair to compare the extrapola-

TABLE V

The volume of distribution of mannitol in two individuals obtained by using three different single injection methods (data from literature—Elkinton (13))

Subject weight	Time after injection of mannitol	Volume of distribution of mannitol						
		Simple dilution method*		Extrapolation method		Constant-change method		
						Time interval		
Kg.	*min.*	*Liters*	*% BW*	*Liters*	*% BW*	*min.*	*Liters*	*% BW*
RE-1	30	12.0	17.1					
70.17	60	13.1	18.7			30–60	8.9	12.6
	120	16.5	23.5			60–120	9.0	12.8
	180	17.6	25.1	12.9	18.4	120–180	14.7	21.0
RE-2	30	12.2	17.5					
69.85	60	13.5	19.3			30–60	8.5	12.6
	90	13.6	19.5			60–90	13.8	19.7
	120	13.3	19.0			90–120	15.9	22.8
	180	16.0	22.9	14.1	20.4	120–180	14.4	20.7
RE-3	30	12.1	17.1					
70.78	60	13.6	19.2			30–60	8.3	11.7
	90	14.4	20.3			60–90	10.4	14.7
	120	14.6	20.6			90–120	13.4	18.9
	180	14.6	20.6	14.8	20.9	120–180	14.6	20.7
LG-1	30	13.9	20.5					
67.3	60	15.4	22.8			30–60	9.6	14.2
	90	15.3	22.6			60–90	16.1	23.8
	120	15.3	22.6			90–120	16.4	24.2
	180	15.3	22.6	17.3	25.6	120–180	14.9	22.1
LG-2	30	14.2	20.8					
68.46	60	15.7	23.0			30–60	10.2	14.9
	90	16.0	23.4			60–90	14.7	21.5
	120	16.0	23.4			90–120	15.9	23.2
	180	16.8	24.6	17.0	24.8	120–180	14.6	21.4

* Calculated by Elkinton.

tion and constant-change methods on the basis of data presented in Tables II and III since the extrapolation, in some experiments, did not meet the requirements of the method as described by Cardozo and Edelman (6).

As indicated by Table IV, in all experiments the constant-infusion method gave smaller values for the distribution of sucrose, as well as mannitol, than the constant-change method. A possible reason for this is that, after the constant infusion was stopped, all of the indicator in the animal was not recovered. Deane (8) states that for inulin the urine should be collected for at least 5 hours in dog, after the infusion is discontinued. It would appear that the 6-hour collection period used in these experiments should have been sufficient for recovering all of the sucrose and mannitol, since these substances have a molecular weight smaller than that of inulin. It may be that all of the injected sucrose and mannitol could never be recovered.

The volume of distribution of inulin in man, estimated by the constant-infusion method, has been reported as representing 16 per cent of body weight (14). The mean value obtained by the constant-change method was 18.7 per cent (Table II). Again, it appears likely that all of the injected inulin was not recovered in the infusion method. In man, the constant-infusion method requires 6 hours of inulin infusion plus 18 to 24 hours of urinary collection, whereas the constant-change method requires about 4½ hours, if the longest time interval is used. The fact that the indicator is rapidly excreted by glomerular filtration is an advantage in the constant-change method, but a disadvantage in the infusion method (2, 5). The time required to determine the volume of distribution of a substance by the constant-change method is shortened if the substance diffuses and is excreted more rapidly.

The observation that the volume of distribution of sucrose is about 80 to 90 per cent of the man-

nitol distribution in intact animals, confirms the work of Swan, Madisso, and Pitts (12) who worked with nephrectomized dogs. Moreover, the values obtained for the distribution of sucrose and mannitol as a per cent of body weight compared fairly well. (*Sucrose:* Intact animals; mean 21.6 and range 20.7 to 22.2 per cent. Nephrectomized; mean 21.3 and range 18.8 to 25.0 per cent. *Mannitol:* Intact animals; mean 24.9 and range 24.6 to 25.5 per cent. Nephrectomized; mean 22.6 and range 18.7 to 27.2.) The question of whether or not the volume of distribution of inulin, sucrose, or mannitol is a measure of the extracellular fluid in intact animals cannot be answered by the present investigation.

2. *An analysis of the basic assumptions*

The first, implicit, assumption is that the body fluid compartment which is being measured does not change with time, *during the period of measurement.* How long the body fluid compartment must remain constant depends upon the indicator. A short time interval could be used if the concentration of the indicator could be very accurately determined. If a short time interval were used with inulin, for example, the error of the chemical determination of the substance would unduly influence the result. So, with inulin a long time interval is desirable and the body fluid compartment would have to remain constant during the period of observation. Perhaps a radio-active indicator, which avoids dealing with plasma and urine "blanks" and which can be accurately measured, may allow the use of relatively short intervals of time.

If one were able to use Equation (*5*), no other assumptions would be necessary. However, Equation (*5*) cannot be used directly, and certain simplifying assumptions must be made in order to obtain a useful Equation (*8*). The second assumption is that, for a two volume system such as the extracellular fluid, the difference between the interstitial and plasma indicator concentrations remains constant, or very nearly constant, *during the period of measurement,* so that the term, $V_2 \frac{d}{dt} (C_2 - C_1)$, of Equation (*5*) can be eliminated. This condition of constant difference, during short intervals of time, is approached some time after point P shown in Figure 2 (using inulin as an example). The third assumption is that, *during the period of measurement,* the plasma concentration of the indicator and the urinary excretion of the indicator versus time curves approach a straight line. The three assumptions are interrelated and depend a great deal upon the indicator, which more than anything else, determines the length of the measurement period. All three assumptions become more valid as the period of measurement is shortened.

3. *Steady state conditions*

The above assumptions define the steady state conditions which should exist, or be approached, after a substance is introduced into the body, if the constant-change method is to be used for determining the volume of distribution of that substance. It is felt that, following a single intravenous injection of an indicator, a steady state exists when the value of the distribution of the substance, calculated on the basis of many time intervals, remains relatively constant.

4. *Ideal substance*

In order to use the method of constant-change, an ideal substance should possess the following properties: 1) It should be distributed in the body fluid volume which is to be measured. 2) It should diffuse *and be excreted in the urine* rapidly. A rapid change in plasma concentration of the indicator is desirable, since a shorter period of time would be required to measure significant differences in plasma concentration and urinary excretion of the indicator. Many observations could be made over a short period of time, so that numerous confirmatory calculations of the volume of distribution of the indicator could be obtained. 3) During the period of measurement, the extrarenal loss of the indicator should be insignificant. The total amount of indicator which leaves the volume, prior to measurement, is unimportant. 4) It should not change the body fluid volume which is to be measured. 5) It should not be toxic. 6) It should be determined accurately.

SUMMARY

1. A constant-change, single injection method is presented for estimating the apparent volume of

distribution of substances in body fluid compartments of intact animals. For a two compartment system, the extracellular space for example, the apparent volume of distribution of an indicator equals the urinary excretion of the indicator during a certain time interval divided by the change in plasma indicator concentration during the same time interval. Theoretical and experimental justification is presented for selecting and identifying the proper time interval: 1) the volume of distribution of the indicator should not change during the period of measurement, 2) the difference in interstitial and plasma indicator concentrations should remain constant, or very nearly constant, 3) during the time interval, the plasma concentration of the indicator and the urinary excretion of the indicator versus time curves should approach a straight line. The limitations of the basic assumptions, used in deriving the above equation, are analyzed. The basic assumptions define equilibrium conditions which should exist, if the constant-change method is to be used. For best results the method should be utilized with a substance which has the following ideal properties: 1) It should be distributed in the body fluid volume which is to be measured. 2) It should distribute and be eliminated from the body rapidly. 3) During the period of measurement, the extrarenal loss of the indicator should be insignificant. 4) It should not change the body fluid volume which is to be measured. 5) It should not be toxic. 6) It should be determined accurately.

2. The constant-change method is compared with the simple dilution, extrapolation, and constant-infusion methods and found to have the following advantages: 1) It is independent of the amount of indicator used, and 2) prior to the period of measurement, it is unrelated to the amount of indicator lost from the fluid compartment in which the indicator is distributed. 3) The indicator need not be completely excreted before the distribution of a substance can be determined following a second injection. 4) It offers the possibility of estimating small changes in the distribution of the indicator. 5) It requires that only the relative change in plasma concentration of the indicator be measured.

3. The volume of distribution of inulin averaged 18.7 per cent of body weight in 6 normal individuals. (Range 16.0 to 20.0 per cent and standard deviation ± 1.57 per cent.)

4. The volume of distribution of sucrose, as a per cent of the mannitol distribution, averaged 84.9 (80.2 to 90.2) in four dogs.

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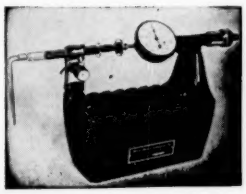

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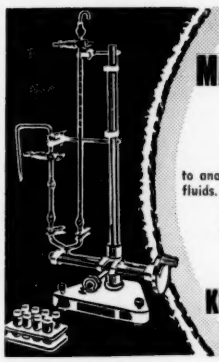

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